Purinergic Signalling (2008) 4:213–236
DOI 10.1007/s11302-008-9104-4

ORIGINAL ARTICLE

Purinergic receptors and gastrointestinal secretomotor function

Fievos Leontiou Christofi

Received: 17 May 2007 / Accepted: 7 April 2008 / Published online: 5 July 2008

Abstract Secretomotor reflexes in the gastrointestinal (GI) tract are important in the lubrication and movement of digested products, absorption of nutrients, or the diarrhea that occurs in diseases to flush out unwanted microbes. Mechanical or chemical stimulation of mucosal sensory enterochromaffin (EC) cells triggers release of serotonin (5-HT) (among other mediators) and initiates local reflexes by activating intrinsic primary afferent neurons of the submucous plexus. Signals are conveyed to interneurons or secretomotor neurons to stimulate chloride and fluid secretion. Inputs from myenteric neurons modulate secretory rates and reflexes, and special neural circuits exist to coordinate secretion with motility. Cellular components of secretomotor reflexes variably express purinergic receptors for adenosine (A1, A2a, A2b, or A3 receptors) or the nucleotides adenosine 5′-triphosphate (ATP), adenosine diphosphate (ADP), uridine 5′-triphosphate (UTP), or uridine diphosphate (UDP) ($P2X_{1\text{-}7}$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$ receptors). This review focuses on the emerging concepts in our understanding of purinergic regulation at these receptors, and in particular of mechanosensory reflexes. Purinergic inhibitory (A_1, A_3, $P2Y_{12}$) or excitatory (A_2, $P2Y_1$) receptors modulate mechanosensitive 5-HT release. Excitatory ($P2Y_1$, other P2Y, P2X) or inhibitory (A_1, A_3) receptors are involved in mechanically evoked secretory reflexes or "neurogenic diarrhea." Distinct neural (pre- or postsynaptic) and non-neural distribution profiles of $P2X_2$, $P2X_3$, $P2X_5$, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, or $P2Y_{12}$ receptors, and for some their effects on neurotransmission, suggests their role in GI secretomotor function. Luminal A_{2b}, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are involved in fluid and Cl^-, HCO_3^-, K^+, or mucin secretion. Abnormal receptor expression in GI diseases may be of clinical relevance. Adenosine A_{2a} or A_3 receptors are emerging as therapeutic targets in inflammatory bowel diseases (IBD) and gastroprotection; they can also prevent purinergic receptor abnormalities and diarrhea. Purines are emerging as fundamental regulators of enteric secretomotor reflexes in health and disease.

Keywords Chloride secretion · Gastrointestinal tract · Enterochromaffin cells · 5-HT release · Submucous plexus · Epithelial cells · Mucosal reflex · Mechanosensitivity · Purinergic receptors · P2X receptors · P2Y receptors · Adenosine receptors

Abbreviations

5-HT	serotonin
ADA	adenosine deaminase
AK	adenosine kinase
AR-C69931MX or cangrelor	*N*6-(2-methylthioethyl)-2-(3,3,3-trifluoropropylthio)-βγ-dichloromethylene-ATP
ATP	adenosine 5′-triphosphate
CaCC	Ca^{2+}-dependent Cl^- channels
cAMP	3′,5′-cyclic adenosine monophosphate

F. L. Christofi
Department of Anesthesiology, The Ohio State University,
Columbus, OH 43210, USA

F. L. Christofi (✉)
College of Medicine and Public Health,
The Ohio State University,
226 Tzagournis Medical Research Facility,
420 West 12th Avenue,
Columbus, OH 43210, USA
e-mail: christofi.1@osu.edu

CFTR	cystic fibrosis conductance transmembrane regulator
ChaT	choline acetyltransferase
EC	enterochromaffin cell
ENaC	amiloride-sensitive epithelial Na^+ channels
ENS	enteric nervous system
EPAN	extrinsic primary afferent neuron
GI	gastrointestinal tract
IBD	inflammatory bowel diseases
IPAN	intrinsic primary afferent neuron
-ir	immunoreactivity
Isc	short-circuit current indicative of chloride secretion
2MeSADP	2-methylthioADP
MRS2279	2-chloro-*N*6-methyl-(*N*)-methanocarba-2′-deoxyadenosine-3′5′-bisphosphate
MRS2179	2′-deoxy-*N*6-methyladenosine-3′5′-bisphosphate
MRS2211	6-(2′-chloro-5-nitro-azophenyl)-pyridoxal-α5-phosphate
MRS2567	1.2-di-(4-isothio-cyanatophenyl) ethane
NBTI	*S*-(p-nitrobenzyl)-6-thioinosine
NPY	neuropeptide Y
PLC	phospholipase C
PPADS	pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid) tetrasodium salt
R	receptor
SP	substance P
VIP	vasoactive intestinal peptide

Introduction

Gastrointestinal (GI) secretions in coordination with motility are important in digestion of food particles, lubrication, nutrient absorption, regulation of pH and solute concentration, and elimination of waste products. Diarrhea occurs in GI diseases to flush out noxious chemicals or unwanted microbes. Intestinal chloride secretion provides a driving force for fluid movement and obligate transport of water into the lumen. Under normal circumstances, mucosal chloride secretion is very low, but can reach extraordinary rates when challenged by enterotoxins or various secretogogues. Hallmarks of enteric infections or inflammatory bowel diseases (IBD) are malaise and diarrhea; the other extreme is constipation that occurs in patients with idiopathic constipation, constipation-predominant irritable bowel syndrome (IBS), or opioid-induced constipation. Structural or functional changes in the enteric nervous system (ENS), in neurotransmitters, signaling pathways, or other components of enteric neural reflexes may be the basis for the pathogenesis of disturbances in gut motor function.

The current review will focus primarily on the role of purines in secretomotor function in the GI system. The enterochromaffin–neural circuit–epithelial reflex pathway is our target for understanding secretomotor function in the intestinal tract. All cellular components of mucosal reflexes express purinergic receptors, and this review will focus on these receptors, their distribution, and function in mucosal reflexes and secretomotor function in the GI tract. The enteric neural circuits and cellular components involved in neurosecretory reflexes will first be briefly reviewed. A brief overview and basic knowledge of purinoceptor classification, pharmacology, receptor ligands and their selectivity is necessary in order to understand the concepts forwarded in this review. Emerging concepts will be reviewed in our understanding of purinergic regulation in mucosal reflexes, with particular emphasis on enterochromaffin cells (EC), mucosal reflexes, receptor distribution, and luminal P2 receptors involved in epithelial ion transport and fluid secretion. In addition, we review recent findings on purinergic regulation of gastric secretions, and in relation to gastric mucosal protection. Our review would not be complete without a very brief description of the emerging role of purinergic receptors as therapeutic targets in IBD [2] or implications of purine receptor abnormalities (up- or downregulation) in GI diarrhea diseases. The reader is also referred to more general reviews on purinergic signaling [12], GI secretomotor function and dysfunction [25, 26], epithelial secretion, and hepatobiliary function [92]. Other relevant reviews include adenosine neuromodulation [20], purines in mechanosensory signaling [26], ATP as a sensory transmitter [6], and $P2Y_1$ receptors in secretomotor function [113], or P2X receptors as potential targets in IBS [40].

Enterochromaffin–neural–epithelial reflex pathways

The secretory reflex is composed of sensory EC cells, intrinsic primary afferent neurons (IPANs), various interneurons, secretomotor neurons, interplexus neurons for coordinating secretion and motility or for secretomotor function [11], and the epithelial cells involved in ion transport and fluid secretion in the gut lumen (Fig. 1). Luminal stimulation by nutrients, changes in pH, mechanical forces, changes in solute concentration, luminal irritants, or invading enteropathogenic microorganisms will activate the EC cells to secrete serotonin (5-HT) among

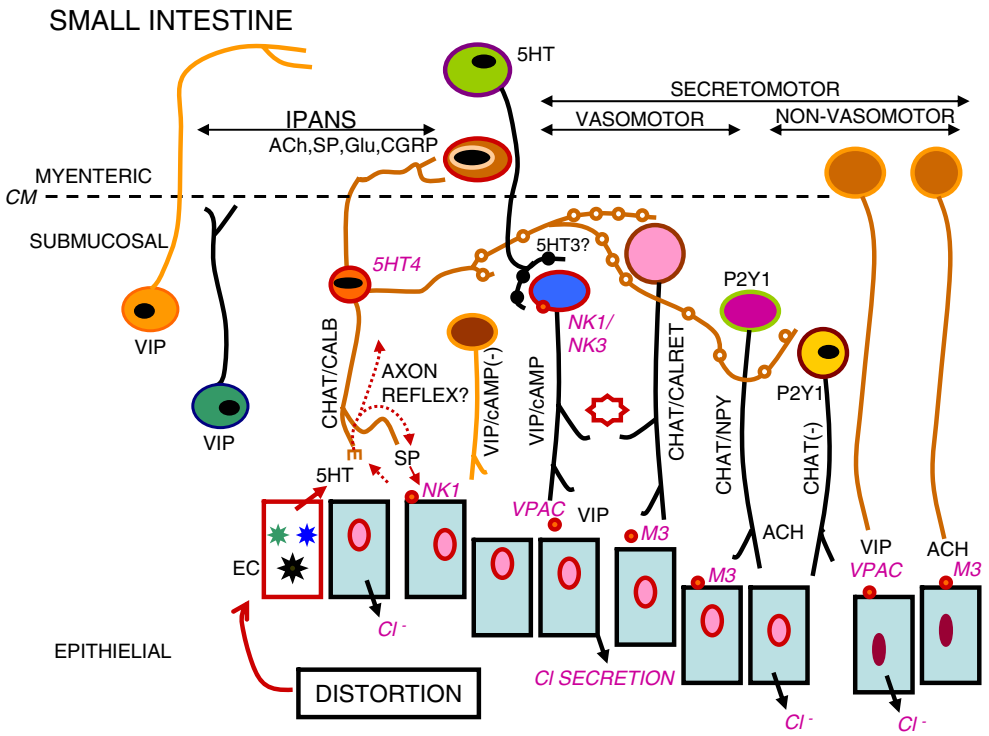


Fig. 1 Generic intestinal secretory reflex based on guinea pig intestine. Neural reflex pathway regulating secretion includes EC sensory cells that release 5-HT to activate IPANS projecting to the mucosa, to submucous ganglia, and to myenteric ganglia. IPANS synapse with several types of cholinergic (*CHAT*) and vasoactive intestinal peptide (*VIP*) secretomotor neurons and release acetylcholine (*ACh*) at muscarinic receptors (*M3*) and VIP at VIP receptors (VPACRs) on epithelial cells, or arterioles. VIP neurons are subdivided according to their ability to generate cAMP as VIP/cAMP (+) and VIP/cAMP (-) and may be further subdivided into VIP/cAMP/A2aRs and VIP/cAMP/A2aR (-). Cholinergic neurons are ChAT/calretinin (CALRET), CHAT/NPY, CHAT (-). Mechanical distortion such as brush stroking the mucosa is one stimulus for activation of the reflex. Myenteric secretomotor neurons project to the mucosa as well. *CGRP* calcitonin gene-related peptide, *EC* enterochromaffin cells, *5-HT* 5-hydroxytryptamine, *NK1* neurokinin-1, *NPY* neuropeptide Y, *SP* substance P, *A2aRs* adenosine A_{2a} receptors. (Modified from [25] by permission)

other mediators to trigger secretomotor reflexes leading to chloride, bicarbonate, potassium, mucin, and fluid secretion. EC cells communicate with both submucosal and myenteric IPANS to initiate secretomotor reflexes. Mucosal stimulation and activation of secretomotor reflexes via long myenteric pathways may play a role in the coordination of intestinal motility, secretion, and blood flow [88]. This review is restricted to submucous pathways and secretomotor reflexes. Under physiological circumstances, large fluctuations in secretions can occur depending on the stimulus. Crohn's disease or *Clostridium difficile* toxin A (TxA) can cause diarrhea and alterations in secretion. However, the diarrhea observed in IBD is mainly malabsorptive not secretory; in the inflamed gut, secretion actually decreases. Hypersecretion due to infection is helpful in flushing to rid the lumen of a pathogen.

Release of sensory mediators from EC cells activates the afferent nerve endings of IPANs to trigger a secretory reflex. IPANs detect sensory information from the luminal environment in the form of action potentials carried in their primary afferent process. The sensory information is decoded and integrated in the cell soma and then relayed to interneurons or secretomotor neurons to elicit a secretory reflex. These IPANs in submucous ganglia have AH cell electrophysiological characteristics and comprise 15% of submucous neurons (and 25-30% of myenteric neurons). The axonal processes of submucous IPANs are known to project to other IPANs and to submucous or myenteric neurons. They have Dogiel type II morphology and project in all directions in the submucous plexus [25].

One type of IPAN in the submucous plexus has one sensory process projecting to the mucosa and responds to mucosal distortion/deformation at sites less than 1 mm^2 from the recording site. Another type of IPAN in the submucous plexus is sensitive to distention [26, 38, 112]. Notable differences exist in the chemical coding of these neurons in human, porcine, and rodent intestine [25]. After activation of IPANs, release of transmitters at synapses with interneurons or secretomotor neurons leads to epithelial secretion. 5-HT interplexus neurons send a process to synapse with secretomotor neurons expressing 5-HT3 receptors. Secretomotor neurons are S/type 1 neurons that

release acetylcholine or VIP to activate the chloride-secreting crypt epithelial cells expressing muscarinic and VIP receptors. All cellular components of this reflex have functional adenosine (P1) or nucleotide (P2X and P2Y) receptor subtypes and are the subject of this review.

Purinergic receptors

Adenosine, adenosine 5′-triphosphate (ATP), ADP, AMP, uridine 5′-triphosphate (UTP), UDP, and UDP-glucose are endogenous purines that activate P1, P2X, or P2Y purinoceptor families that are widely and differentially distributed in the ENS and non-neuronal cells in the GI tract. The tissue distribution and/or biological experiments suggest that up to 14 of 18 purinoceptors may be involved in secretomotor reflexes in the GI tract. Still, our knowledge remains incomplete.

Adenosine interacts with four cell surface P1 receptors, designated as A_1, A_{2a}, A_{2b}, and A_3 receptors to influence a variety of physiological functions. The endogenous ligand is adenosine (or AMP, [41]). Receptor classification is based on receptor cloning, pharmacological studies, and mouse receptor knockout models. All four receptors are expressed in the GI tract, but their characterization is incomplete (see later). Adenosine receptors belong to the superfamily of seven transmembrane domain G protein-coupled receptors (GPCR) and they are emerging as potential therapeutic targets [53].

The nomenclature for the transmitter-gated ion channel $P2X_{1\text{-}7}$ receptors relies mostly on recombinant receptor pharmacology as reviewed by Khakh et al. [57]; the pharmacology of the P2X receptors in whole tissues is complicated by species differences, marked ectonucleotidase activity that interferes with agonist/antagonist potency profiles, and lack of very selective ligands for P2X receptor subtypes. Their review covers the functional properties of recombinant receptors, their pharmacology, ion effects (Zn^{2+}, H^+, Ca^{2+}), and some native receptors in tissues.

A recent review summarizes the pharmacological profiles of cloned mammalian metabotropic P2Y receptors [110] that have been shown to mediate actions of nucleotides when expressed in functional systems. Eight P2Y receptors have been cloned and defined functionally. The $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors are coupled to stimulation of phospholipase C (PLC). $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors are negatively coupled to adenylyl cyclase (AC); the $P2Y_{11}$ receptor is positively coupled to AC. Abbrachio et al. [1] provide an update on molecular mechanisms, pathophysiology, and therapeutics of P2Y receptors.

$P2Y_1$ receptors are involved in platelet aggregation, vasodilation, neuromodulation, mechanosensitivity, and secretomotor reflexes. The $P2Y_1$ receptor is activated by ADP or its analog 2-methylthioADP (2MeSADP). A potent and selective antagonist is 2′-deoxy-*N*6-methyladenosine-3′5′-bisphosphate (MRS2179) or MRS2279. Adenosine 5′-*O*-(1-boranotriphosphate) (ATP-α-B) derivatives are novel $P2Y_1$ receptor agonists that may be of potential clinical relevance [78].

The $P2Y_2$ receptor plays an important role in Cl^- secretion from epithelial cells, and a $P2Y_2$ receptor agonist diquafosol is used for treatment of dry eye disease. It is activated by UTP and ATP and their actions are blocked by suramin, although suramin has affinity for non-purinergic receptors as well [54]. The $P2Y_4$ receptor is expressed in epithelia, and depending on the species, the receptor has a strong preference for UTP (human receptor) or equal preference for UTP and ATP (rat receptor). Unlike the $P2Y_2$ receptor it is not blocked by suramin. The $P2Y_6$ receptor is distributed in the cardiovascular system and brain. It has a preference for UDP and 1,2-di-(4-isothiocyanatophenyl)ethane (MRS2567) is a selective antagonist. UDP also acts on receptors for cysteinyl leukotrienes. The $P2Y_{11}$ receptor is blocked by suramin (and reactive blue 2) and it prefers ATP as an agonist. $P2Y_{12}$ and $P2Y_1$ receptors are similar in that they are both activated by ADP and more potently by 2MeSADP. $P2Y_{12}$ receptor antagonists are effective in clinical use to inhibit platelet aggregation; the receptor is also involved in inhibition of neuronal cells. Antagonists include *N*6-(2-methylthioethyl)-2-(3,3,3-trifluoropropylthio)-βγ-dichloromethylene-ATP (AR-C6931MX or cangrelor), AZD6140, and active metabolites of clopidogrel and prasugrel. The $P2Y_{13}$ receptor is found in nerve cells and immunocytes and is activated similar to the $P2Y_{12}$ receptor, but is selectively blocked by 6-(2′-chloro-5-nitro-azophenyl)-pyridoxal-α5-phosphate (MRS2211). The $P2Y_{14}$ receptor is activated by UDP-glucose.

Sensory enterochromaffin cells

5-HT release from EC cells activates secretory, peristaltic, and vasomotor reflexes and contributes to the coordination of these reflexes. As shown in Fig. 2, EC cells with long basally located fine projections containing serotonin and sometimes connecting to neuron-like structures could be observed; other cells had short blunt processes or were dividing EC cells. A provocative possibility is the recent speculation that EC cells may even function as primary neurons [44].

EC cell dysfunction is implicated in malignant carcinoid, dumping, and irritable bowel syndromes, as well as IBD. 5-HT secretion from carcinoid tumors is one of the putative mediators of carcinoid diarrhea that can be relieved by 5-HT antagonists. 5-HT3 antagonists or a partial 5-HT4 agonist (i.e., tegaserod) are beneficial in treating diarrhea and constipation predominant symptoms in patients, respectively

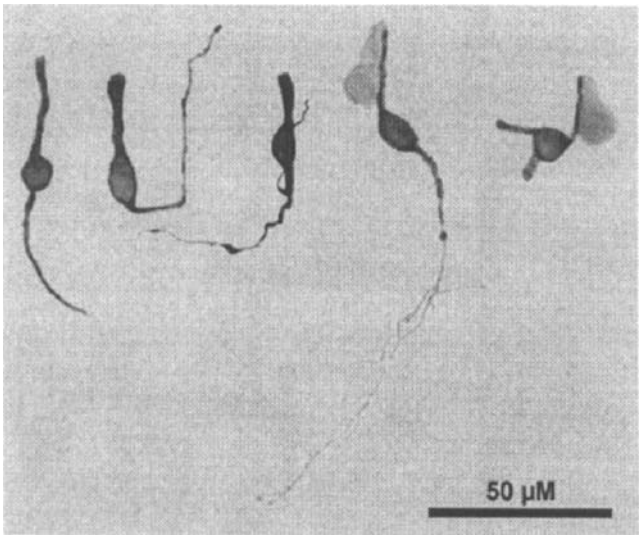


Fig. 2 Photomicrographs showing serotonin immunolabeled, formalin-fixed EC cells dispersed from the colonic mucosa. All cells have luminal projections. From the *left*, four serotonin-positive cells with one or two basally located, axon-like projections are seen. The fourth of these cells connects with a neuron-like structure. *Right*: a cell with short and blunt basal projections. The EC cells are in some cases attached to neighboring mucosa cells. (Modified from [44] by permission)

[4, 67, 97]. Mucosal defects in 5-HT signaling occur in ulcerative colitis, diarrhea (or constipation) predominant IBS [24] or experimental colitis [67]. A better understanding of the signaling mechanisms, function, and dysfunction of EC cells is essential in understanding gut reflexes and symptoms of several diseases.

Despite the obvious importance of EC cells and 5-HT release in normal or disease states, our understanding of purinergic or other mechanisms involved in the regulation of 5-HT release is limited. Tissue studies are of limited use in helping us understand the complex regulation of 5-HT release because of potential interference from mediators released from neighboring cells. The human carcinoid BON cell line is a suitable EC cell model to study receptor and intracellular signaling pathways regulating 5-HT release [34].

A clone of BON/EC cells that is 99% enriched in 5-HT is a suitable EC model for population responses or for single cell functional studies. BON cells retain many of the mechanosensitive and chemosensitive properties of non-transformed cells, they respond to nutrients (glucose), mechanical activation (shaking, touch, stretch, volume changes, pressure), as well as receptor activation (purinergic, muscarinic, neurotensin, 5-HT, VIP, adrenergic, somatostatin, cholecystokinin, dopamine, bradykinin, etc.). A comparison of EC and BON cells indicates similarities in expression of receptors, ion transporters, ion channels, chemical mediators, or other mechanisms reviewed by Cooke and Christofi [25]. In addition to 5-HT, they secrete or contain adenosine, ATP neuron-specific enolase, synaptophysin, chromogranin A, neurotensin, vasoactive intestinal peptide (VIP), prostaglandins, and others. BON cell injection into nude mice can also serve as a model of carcinoid tumors [35].

Chemosensitivity of EC cells

EC cells have microvilli protruding into the lumen raising the possibility that they may "taste" the luminal contents. The nutrient monosaccharide D-glucose (10-100 mM) or the nonmetabolizable α-D-glucopyranoside (not fructose) caused a graded increase in 5-HT release in BON cells that was not caused by changes in osmolarity. Phloridzin blocked the effect of D-glucose by blocking D-glucose uptake [59, 87]. The role of purinergic modulation of nutrient activation of 5-HT release remains unknown.

Mechanosensitivity of EC cells

Mechanosensitivity in EC cells is poorly understood and the mechanosensitive elements remain unknown. Brush stroking, compression of the mucosa or villi, rotational stroking, or pressure/volume changes used to evoke mucosal secretomotor reflexes in the gut are not suitable to elicit 5-HT release in isolated EC/BON cells. Mechanical forces in the gut are complex and likely represent a composite of forces (pressure, shear force, centrifugal force, stretch, deformation, compression, touch, etc.). We succeeded in employing three different mechanical stimuli to elicit responses in EC cells to study purinergic regulation of mechanosensitive 5-HT release. Rotational shaking of cultured cells simulates forces that are generated in the intestinal lumen (i.e., shear forces, centrifugal forces, changes in hydrostatic pressure) and releases 5-HT. Pressures of 75 mmHg or greater (normally associated with painful stimulation) applied to cells in a pressure chamber or laminar shear stress of 1–2 dyne/cm^2 applied in a parallel plate flow chamber evoked 5-HT release [26].

Second messengers in mechanosensitivity

Balloon distension and puffs of nitrogen gas/pressure stimulate 5-HT release and trigger a reflex secretory response via submucous neurons in intact mucosa/submucosa preparations [84]. Rotational shaking of tissue preparations or the EC/BON cells in culture predominantly activates a Gαq/PLC/inositol triphosphate (IP_3)–Ca^{2+} signaling pathway leading to 5-HT release [59]. A separate Gs/Gi/AC/

PKA (protein kinase A)/cAMP signaling pathway provides a minor contribution to mechanosensitive 5-HT release [18].

Dual modulation of mechanosensitivity by $P2Y_1$ and $P2Y_{12}$ receptors

Recent evidence from pharmacological and molecular studies support the hypothesis that dual modulation of 5-HT release occurs via excitatory $P2Y_1$ receptors coupled to the Gαq/PLC/IP_3-Ca^{2+} signaling pathway leading to 5-HT release [114] and inhibitory $P2Y_{12}$ receptors coupled to AC (Christofi, Wunderlich, Xue, Cooke, unpublished observations; [120]). Touch/stretch evokes a Ca^{2+} response in BON/EC cells that is used to study purinergic regulation of mechanosensitivity. In preliminary studies, touch Ca^{2+} responses in EC cells were inhibited by apyrase (ectonucleotidase) whereas a 5′-ectonucleotidase inhibitor ARL 67156 augmented responses indicating that a nucleotide is involved in mechanosensitivity. When single cells are examined and analyzed according to inhibition or augmentation of the Ca^{2+} response, dual modulation of the response is revealed in subsets of EC cells. Therefore, the $P2Y_1$ receptor antagonist MRS2179 could either abolish the touch Ca^{2+} response or augment the response in different subsets of EC cells. MRS2179 could block touch-evoked Ca^{2+} responses in cells responsive to the $P2Y_1$ receptor agonist 2MeSADP. The Ca^{2+} response evoked by 2MeSADP was either inhibited or augmented by MRS2179 in subsets of EC cells; an additional component of the touch Ca^{2+} response was blocked by the P2 receptor antagonist PPADS [114].

In molecular signaling studies, when the human $P2Y_1$ receptor was overexpressed in BON cells with naitive P2Y receptors, it caused additional mobilization of Ca^{2+} in the cells and augmented 5-HT release. A $P2Y_1$ receptor silencing RNA reduced receptor expression leading to a reduction in 5-HT release. In heterologous 1321N1 cells, transfection of human $P2Y_1$ receptors enabled the cells to generate a Ca^{2+} response to mechanical stimulation. $P2Y_1$ receptor antagonists MRS2179 or A3P5P abolished the Ca^{2+} response in 1321N1 cells or 5-HT response in BON cells that overexpressed the human $P2Y_1$ receptor [120]. Overexpression of the $P2Y_{12}$ receptor in BON cells and/or HEK293 cells nearly abolished mechanically evoked 5-HT release (BON) and cAMP (both cell types). Blockade of $P2Y_{12}$ receptors by 2-MeSAMP augmented the cAMP responses. 5-HT release was augmented after knockdown of $P2Y_{12}$ receptors in BON cells; silencing of $P2Y_{13}$ receptors had no effect. Therefore, molecular studies are consistent with pharmacological studies and suggest that dual purinergic modulation of mechanosensory signaling via $P2Y_1$ and $P2Y_{12}$ receptors (that recognize both MRS2179 and 2MeSADP) leads to 5-HT release in EC cells. The $P2Y_1$ and $P2Y_{12}$ receptors may provide new targets for modulation of mucosal secretory reflexes and are potential therapeutic targets, as shown for platelets [1]. The role of other receptors expressed in EC/BON remains unknown. Messenger RNA transcripts for $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$ exist in BON cells [25, 26].

Adenosinergic modulation of 5-HT release

Endogenous adenosine modulates the basal release of 5-HT from EC/BON cells. Adenosine deaminase caused a dose-dependent increase in 5-HT release (Fig. 3a). The nucleoside uptake inhibitor *S*-(p-nitrobenzyl)-6-thioinosine (NBTI) prevented mechanically evoked 5-HT release (Fig. 3b) by elevating endogenous adenosine (Fig. 3c). Overall, endogenous adenosine is sufficient to provide ongoing inhibition of basal and mechanically evoked 5-HT release. The adenosine A_3 receptor is activated by endogenous adenosine since baseline 5-HT release was augmented by an A_3 receptor antagonist MRS1191 and this was sensitive to an A_3 receptor agonist IB-MECA (Fig. 3d); the A_3 effect was restricted to the Ca^{2+} signaling pathway. The A_1 receptor also contributes, and an A_1 receptor antagonist (CPT), an A_3 receptor antagonist (MRS1191), or adenosine deaminase could also augment the touch/stretch Ca^{2+} response (Fig. 3e). Touch Ca^{2+} responses occur via a Gαq/PLC/IP_3 pathway that is the predominant mechanism in 5-HT release from EC cells. 5′-*N*-methylcarboxy aminoadenosine (MECA) elevated both 5-HT and cAMP levels in BON cells. A_2 receptor antagonists could suppress mechanically evoked 5-HT release indicating the endogenous adenosine can contribute to stimulation of 5-HT release via A_2 receptors. Together, these and other findings indicate that endogenous adenosine can activate inhibitory A_3/A_1 receptor–Gαq/PLC/IP_3–Ca^{2+} and excitatory A_{2a}/A_{2b} receptor–AC/cAMP signaling pathways on EC cells to regulate 5-HT release; A_1, A_{2a}, A_{2b}, and A_3 receptors or mRNA transcripts are present in both BON/EC cells, gastric and intestinal 5-HT-ir carcinoid tumors [18]. Recent isolation of ileal EC cells [58] may permit further study and comparison of purinergic signaling mechanisms in normal and diseased human ileum. Real-time electrochemical detection of local mucosal 5-HT release is also very promising [5].

Intestinal secretory reflexes

Hubel's [51] pioneering spirit and studies demonstrated that electrical field stimulation of tissue setup in Ussing chambers can stimulate transepithelial short-circuit current (Isc) changes indicative of ion transport. His studies

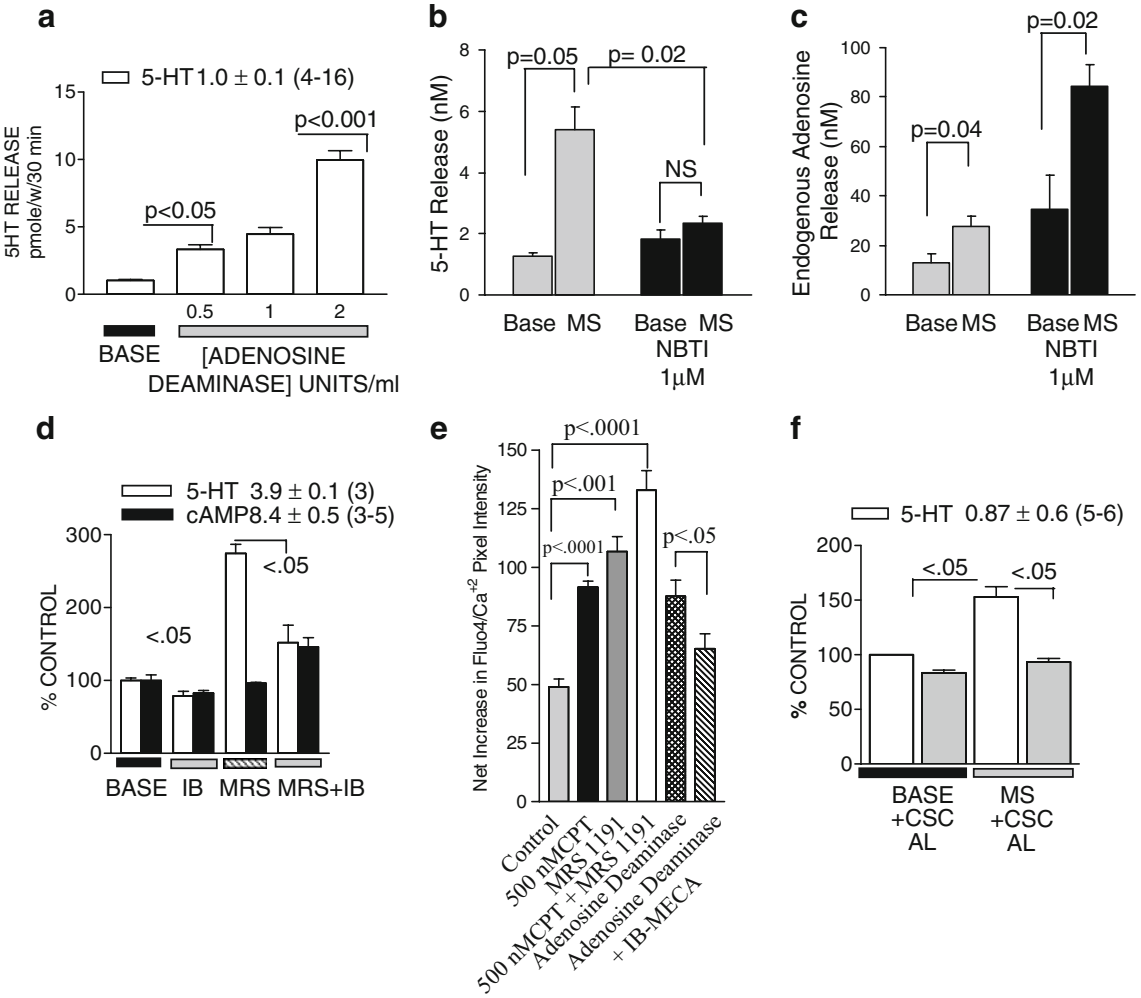


Fig. 3 Regulation of 5-hydroxytryptamine (5-HT) release by endogenous adenosine release from BON cells via inhibitory A_1/A_3 receptors and excitatory A_2 receptors. **a** The enzyme ADA caused a dose-dependent increase in 5-HT release, indicating that release of endogenous adenosine provides an ongoing inhibition of 5-HT release. **b** In the same cells as **a**, NBTI blocked adenosine uptake and abolished mechanically evoked 5-HT release. **c** Adenosine release during resting/unstimulated conditions (*Base*) and mechanical stimulation (*MS*) is augmented in the presence of the adenosine uptake inhibitor *S*-(p-nitrobenzyl)-6-thio-inosine (1 μM NBTI). **d** Chemical stimulation with IB-MECA decreased basal 5-HT and the A_3R agonist cAMP levels. The A_3R antagonist MRS1191 (10 nM) augmented 5-HT release but not cAMP levels ($p<0.05$). The MRS1191-evoked 5-HT response was sensitive to IB-MECA inhibition. **e** Adenosine A_1 and A_3R inhibitory components of touch-evoked Ca^{2+} responses in enterochromaffin/BON cells. The A_3R antagonist MRS1191 augmented the touch Ca^{2+} transient. Chronic exposure to the A_1 antagonist CPT for 24 h caused a significant augmentation in the touch Ca^{2+} response. The stimulatory effects of CPT and MRS1191 were additive on the touch Ca^{2+} response. Adenosine deaminase (5 units/ml)-induced augmentation is reduced by the A_3R agonist IB-MECA (50 nM). **f** A_{2A}/A_{2B} antagonists CGS and AL (alloxazine) inhibited mechanically evoked 5-HT release. Baseline values (pmol/well per 30 min) of 5-HT and/or cAMP are at the *top* of figures with the number of experiments indicated in parentheses. *IB* IB-MECA, *MRS* MRS1191, *ME* MECA, *AL* alloxazine, *CGS* CGS 21680, *MS* mechanical stimulation by rotational shaking at 80 rpm, *NS* not significant. (Modified from [59] by permission)

provided proof that submucous neurons could regulate chloride secretion. This sparked the beginning of a new era of investigation on the influence of the ENS on epithelial secretion and secretomotor reflexes.

The secretory reflex can be activated by nutrients or mechanical stimulation or exposing the gut to food antigens regarded as foreign or to enteropathogenic microorganisms. The mucosal surveillance and immune defense system is exposed to an estimated 10^{12} different antigens per day that mobilizes both intrinsic and extrinsic nervous systems, hormones, paracrine substances, and immune mediators to modulate chloride secretory rates.

The net flux of ions into the gut lumen is the balance of secretion and absorption. Basal secretion or "secretory tone" is also the net flux of ions and fluid into the intestinal lumen that is dependent on the spontaneous release of endogenous paracrine, autocrine, or neurocrine mediators. Secretory tone is assessed from changes in basal Isc, a measure of chloride secretion. The basal Isc is under ongoing neural stimulation. Data with P2 receptor antago-

nists (PPADS, suramin) or P1 receptor antagonists (CPT), nucleotidases, or nucleotidase inhibitors indicated that endogenous nucleotides stimulate, while endogenous adenosine inhibits basal Isc [29]. However, basal secretion is not affected by P2X or $P2Y_1$ receptors because neither of NF279 or MRS2179 influenced Isc. Overall, adenosine, nucleotides, somatostatin, and prostaglandins all contribute to "secretory tone" [26, 27].

$P2Y_1$ receptors and mucosal stroking-evoked neurosecretory reflexes in the colon

Mechanical or chemical activation of the mucosa can lead to an intestinal neural reflex and an increase in ion transport and fluid secretion. Very few studies have investigated purinergic regulation of secretomotor function [18, 27–29]. Mucosal distortion by brush stroking or distension can elicit the reflex (Fig. 4).

Brush stroking of the mucosa causes an intestinal neural reflex response and an increase in Isc indicative of electrogenic chloride ion transport. The brush stroking reflex in the rat colon mucosa-submucosa is reduced by MRS2179 or apyrase and further inhibited by tetrodotoxin (TTX). 2MeSADP, a mixed $P2Y_2/P2Y_4$ agonist UTP, or ATP (P2 receptor agonist) evoked neural and non-neural secretory responses. Mucosal touch/distension evoked fluo-4/Ca^{2+} responses in submucous neurons were also inhibited by apyrase or blocked completely by MRS2179 (Fig. 5); MRS2179 only reduced Isc in stroking reflexes. The potency profile of nucleotides for reducing Isc is different from that for touch Ca^{2+} responses [18]. $P2Y_1$ immunoreactivity was identified in a majority of VIP, NOS, calretinin, NPY, or somatostatin neurons but not SP or calbindin submucous neurons. $P2Y_4$ immunoreactivity occurred mainly in the cell somas of NPY neurons (93%). $P2Y_2$ immunoreactivity occurred in a minority of SP, VIP, NPY CGRP varicose fibers (5-20%) and those surrounding calbindin neurons [18, 26]. Purinergic responses could be distinguished based on stroking reflexes, touch-evoked Ca^{2+} reflexes, actions of nucleotides, or sensitivity to antagonists. These and other data suggest that several nucleotides may contribute to mechanically evoked secretomotor reflexes. Nucleotides differentially activate $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors located at putative pre- or postsynaptic sites on submucous neurons. In rat hippocampus, ATP may activate presynaptic

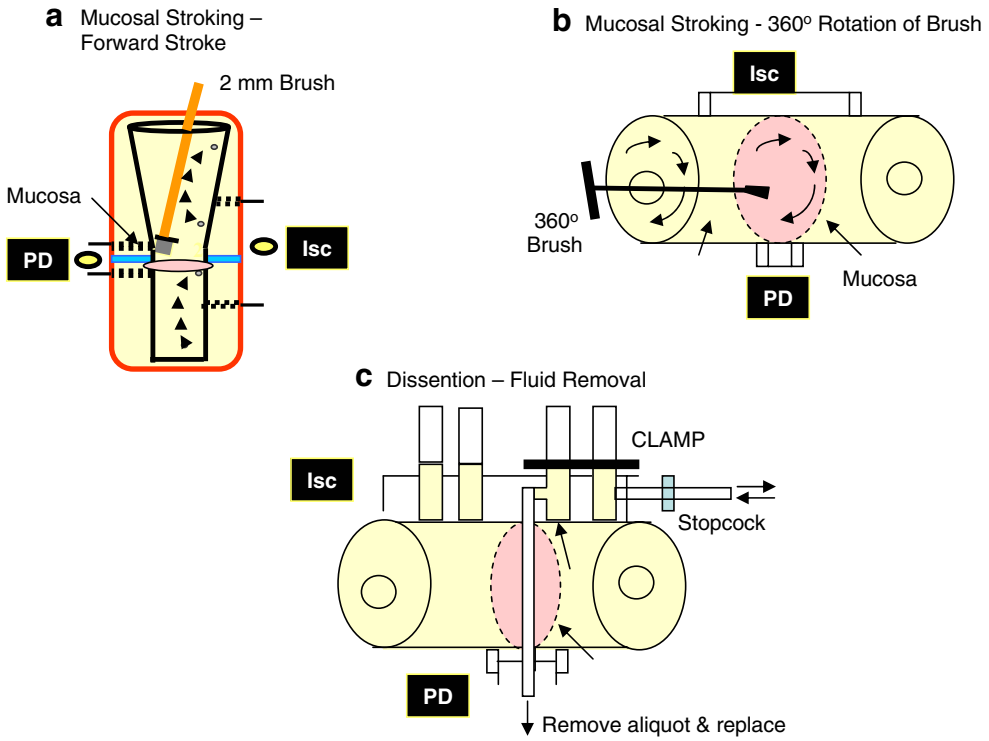


Fig. 4 Modified Ussing chambers used for mucosal stroking studies to evoke a reflex neurosecretory response. **a** Vertical configuration of the chamber permits a 2-mm brush to be placed on the mucosal surface. A single stroke represents the directional movement of the brush from one side to the other on the surface of the mucosa. **b** A complete 360° turn of the brush is another variation for stimulating reflexes; stroke (1 s) is reproducible every 5 min for a 40-min period. **c** Distention is accomplished by removing small volumes of fluid from the serosal side. Drugs are perfused separately on the serosal or mucosal surfaces of the mucosa-submucosa or whole thickness colonic or small bowel tissues. The Isc/short-circuit current nulled out the spontaneous potential difference (*PD*) and was used as a measure of chloride secretion in the distal colon. (Modified from [25] by permission)

$P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors to inhibit glutamate release [91]; presynaptic $P2Y_1$ and $P2Y_2$ receptors also occur in the rat submucous plexus. A working model of the purinergic neural circuitry in the rat colon is shown in Fig. 6.

ARL 67156 augmented, whereas apyrase, atropine, or TTX could inhibit stroking-evoked reflex Isc responses in guinea pig distal colon (Fig. 7a). The agonist potency profile of 2MeSADP > UTP ≥ UDP for stimulation of neural secretion supports a $P2Y_1$ receptor (Fig. 7b), but does not exclude others. MRS2179 reduced the stroking response by 54% and the effect of 2MeSADP by 70%. P2 receptor antagonists PPADS and suramin had additive inhibitory effects. The $P2X_{1/3}$ receptor agonist αβMeATP caused stimulation of Isc, and a desensitizing concentration reduced stroking-evoked secretion but did not affect the 2MeSADP response. Cell bodies of guinea pig colonic submucous neurons expressed P2Y immunoreactivity. About 20-50% of submucous neurons expressed $P2Y_1$ and $P2Y_2$ receptors. P2Y immunoreactivity ($P2Y_1$, $P2Y_2$, or $P2Y_4$) was virtually undetectable in any varicose fibers. $P2Y_1$ immunoreactivity was abundant in putative cholinergic secretomotor neurons and fewer than 2% of NPY/cholinergic secretomotor neurons [71]. In contrast, $P2Y_2$ immunoreactivity was found in all NPY secretomotor neurons and 30% of calbindin/intrinsic sensory neurons. $P2Y_4$ is of minor significance in submucous neurons. In guinea pig, mucosal stroking may activate putative $P2X_{1/3}$, $P2Y_1$, and $P2Y_2$ receptors located at postsynaptic membranes of submucous neurons leading to chloride secretion; $P2Y_1$ receptors on EC also contribute. Based on the available evidence, it was hypothesized that a separate $P2Y_2$ neural circuit pathway exists in guinea pig that is not activated by stroking the mucosa [25, 27]. In both rat and guinea pig, receptors or mRNA transcripts exist for $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{12}$ receptors in the submucosa, and therefore influence of other receptors in stroking reflexes deserves further attention. Species differences exist between mucosal stroking reflexes in rat [18] and guinea pig [27] distal colon in purinergic regulation, and no information exists for the human GI tract.

Neurogenic mucosal secretion and $P2Y_1$ receptors in small intestine

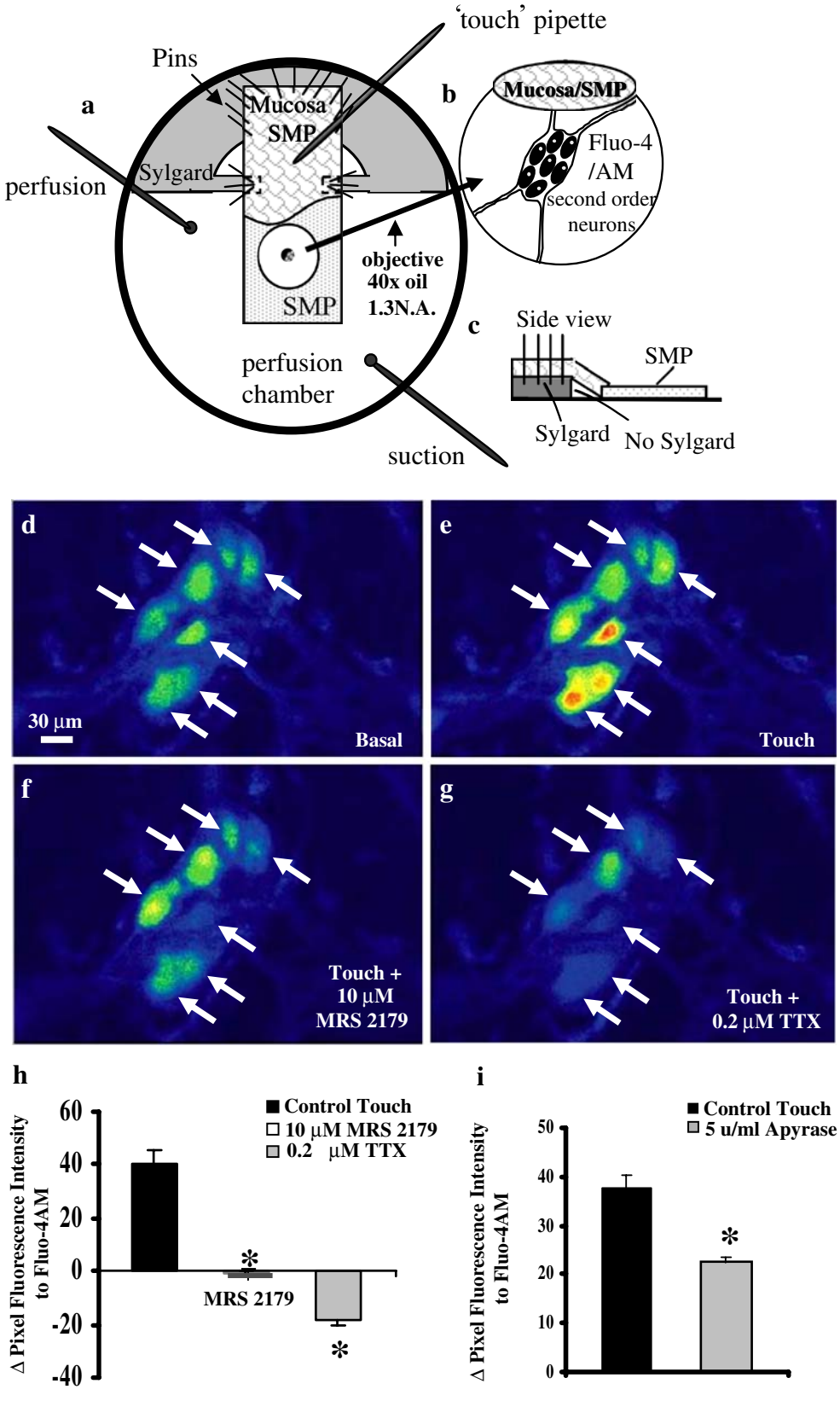
In the guinea pig small intestine, it was shown that neurogenic mucosal secretion is mediated by the $P2Y_1$ receptor that may be expressed on VIP secretomotor neurons. MRS2179 blocked the neurally mediated ATP-evoked response with an IC_{50} of 0.9 μM. TTX could suppress or abolish the ATP response. The $P2Y_1$ receptor was coupled to PLC/IP_3/Ca^{2+}–calmodulin/protein kinase C signaling pathway [37] like that on EC/BON cells.

Fig. 5 Laser confocal imaging of touch-induced purinergic Ca^{2+} signals in neurons of the intact microdissected submucous plexus of the rat distal colon. **a** Perfusion chamber used to record touch-induced Ca^{2+} responses in the neurons. It includes a recording chamber made of a 35-mm microwell dish with a 1-cm, number 0.0 cover glass in the center of the bottom of the dish for visualizing the fluorescent neurons. A portion of the dish is embedded with a 2-mm thick layer of Sylgard shaped around a half moon at one end of the dish. Perfusion with oxygenated buffer or drugs is exactly opposite to the suction. The microdissected M-SMP preparation is stretched and fixed with many (15-20) micropins along the Sylgard layer. The remaining SMP without mucosa is stretched over the glass cover slip of the dish and secured with magnetic metal feet (not shown). A large ganglion of the intermediate layer of the submucous plexus is viewed with a 40× oil immersion objective (N.A. 1.3). **b** Inset shows Fluo-4/AM loaded neurons representing second order neurons that are visualized. **c** Side view of the tissue: The submucous plexus layer is located flat on the bottom of the dish and the mucosa/submucosa lifts over the Sylgard ends at each side of the half moon. The tissue is touched for 20 s on the mucosal surface of M-SMP portion that is stretched across the Sylgard with a fire-polished glass pipette to evoke a Ca^{2+} response in the neurons—the tissue is free to move/stretch downward during the touch, since it is located a distance of 2 mm above the glass bottom. **d–g** The $P2Y_1$ antagonist MRS2179 or TTX abolish the touch-induced Ca^{2+} fluorescence response. A representative example of the effects of MRS2179 and TTX on touch-induced Ca^{2+} transients in a single ganglion (seven neurons in one ganglion). A 20-min exposure to the selective $P2Y_1$ antagonist MRS2179 (10 μM) abolished the touch response in the neurons. In the presence of 0.2 μM TTX, the touch response decreased to below baseline levels. *Arrows* indicate touch-responsive cells. **h** Pooled data for the peak effects of MRS2179 and TTX; averaged transients from 30 neurons from 3 to 4 separate ganglia. **i** Breaking down endogenous nucleotides reduces the touch-induced Ca^{2+} response in the neurons of the intact M-SMP. The touch response was determined in the presence of 5 units/ml apyrase exposed for 10 min. Apyrase reduced by 50% the control touch response (n=4 neurons, p<0.01). Values are means ± SEM. (Modified from [18] by permission)

Purinergic modulation of local inhibitory reflexes

Local inhibitory reflexes to the circular muscle evoked by mucosal application of nutrient amino acids in guinea pig jejunum are partially blocked by antagonists to P2X receptors suggesting involvement of ATP [46]. The receptor subtype(s) have not been characterized. Effects of nutrients on local secretomotor reflexes and involvement of purinergic signaling in the coordination of motility and secretion remain unclear, although some data are emerging on adenosine A_3 and A_1 inhibitory receptors (see later section on "Neurogenic diarrhea"; [8, 9]).

Fluo-4/Ca^{2+} imaging studies are providing new insights into the functional purine receptors involved in the excitability and synaptic transmission in intact neural circuits of the submucous plexus of the human gut. A recent study indicated that the submucous plexus from Roux-en-Y patients is a suitable model to study synaptic transmission in the human enteric nervous system [115]. It was shown that the $P2Y_1$ Gαq-PLC/IP_3 Ca^{2+} signaling


'touch' pipette
Pins
a
Mucosa
SMP
Sylgard
perfusion
objective
40x oil
1.3N.A.
SMP
perfusion
chamber
suction
b
Mucosa/SMP
Fluo-4
/AM
second order
neurons
c
Side view
SMP
Sylgard
No Sylgard
d
30 μm
Basal
e
Touch
f
Touch +
10 μM
MRS 2179
g
Touch +
0.2 μM TTX
h
Control Touch
10 μM MRS 2179
0.2 μM TTX
Δ Pixel Fluorescence Intensity
to Fluo-4AM
60
40
20
0
-20
-40
MRS 2179
*
*
i
Control Touch
5 u/ml Apyrase
Δ Pixel Fluorescence Intensity
to Fluo-4AM
50
40
30
20
10
0
*

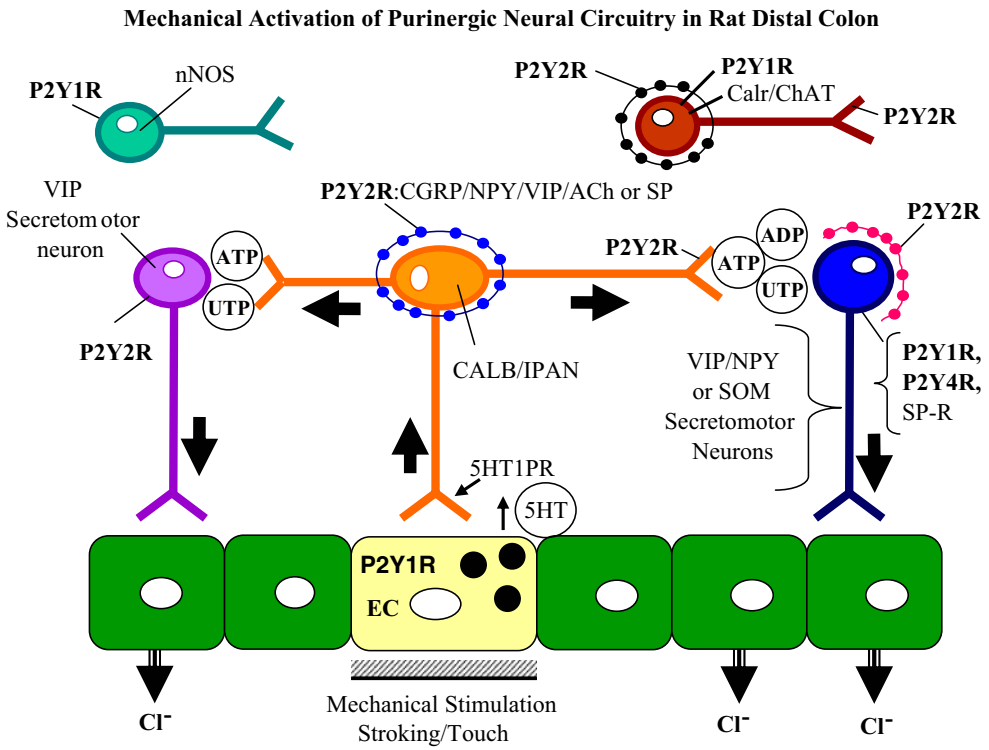


Fig. 6 Mechanical activation of purinergic neural circuitry in rat distal colon. Mucosal brush stroking or touch/stretch releases nucleotides that may include ATP, UTP, and ADP that activate neural $P2Y_1$, $P2Y_2$, and $P2Y_4$Rs at pre- or postsynaptic sites of submucous neurons leading to a net increase in Isc/chloride secretion. Intrinsic primary afferent neurons do not express P2YRs on their cell somas, but receive strong synaptic inputs from extrinsic fibers (i.e., pelvic origin) with $P2Y_2$Rs forming baskets of varicose fibers surrounding their cell somas. A very tiny subset of VIP secretomotor neurons expresses P2Y2R. $P2Y_2$Rs, $P2Y_1$Rs, and $P2Y_4$Rs are coexpressed on >90% of NPY/VIP putative secretomotor neurons that also express SP Rs. $P2Y_1$Rs are also expressed on putative somatostatin (SOM) neurons. Activation of $P2Y_1$Rs leads to a rise in intracellular free Ca^{2+} levels in the secretomotor neurons by ATP, UTP, or ADP. A nicotinic cholinergic synapse has not been ruled out. NOS neurons and CALR/CHAT cholinergic neurons also express $P2Y_1$Rs, but the functional role of these subsets of neurons is unknown. $P2Y_2$Rs are discretely localized on some extrinsic fibers of SP, VIP, NPY, CGRP, or cholinergic origin presumed to be involved in synaptic modulation of transmitter release. (Modified from [18] by permission)

pathway, N-Ca^{2+} channels, nicotinic receptors, and extrinsic nerves are involved in neurotransmission. Pharmacological findings suggest that inhibitory adenosine A_3 receptors modulate nucleotide and cholinergic transmission in the human enteric nervous system.

In the guinea pig small intestine, electrophysiological studies revealed that AMP activates presynaptic inhibitory A_1 receptors to suppress neurotransmission and postsynaptic A_{2a} receptors to elicit a slow excitatory postsynaptic potential (EPSP)-like response. Signaling pathways coupled to A_{2a} receptors include AC, PLC, PKC, and calmodulin-dependent kinases [41]; whether AMP can also activate A_{2b} or A_3 receptors remains unclear.

Endogenous adenosine and A_1 receptors in mucosal reflexes

The role of endogenous adenosine in the physiological regulation of mucosal secretory reflexes was investigated in pharmacological studies [29]. At nanomolar concentrations, the A_1 antagonist 8-chlorophenyltheophylline (CPT) caused a concentration-dependent enhancement of the stroking reflex. The effect still occurred in the presence of an A_{2a} receptor antagonist 8-(3-chlorostyrl) caffeine. Adenosine deaminase (5 U/ml) also enhanced the reflex secretory response, whereas the nucleoside uptake blocker NBTI inhibited it. The A_1 receptor agonist 2-chloro-*N*6-cyclopentyladenosine (CCPA) inhibited reflex-evoked Isc (Fig. 8a; EC_{50}=6 nM) and its effect was also blocked by CPT. In experiments with either piroxicam to block the prostaglandin (PG)-mediated pathway or *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide to block the 5-HT mediated pathway, CCPA reduced or abolished the residual reflex Isc response (Fig. 8b). CCPA could abolish the reflex response to a pulse of 5-HT (Fig. 8c) without affecting TTX-insensitive epithelial responses to carbachol or forskolin. A_1 immunoreactivity was expressed in submucous neurons, in presynaptic varicose nerve endings (synaptophysin-positive neurons), and in glia. The chemical coding of those submucous neurons is not known. Overall, available data suggest that endogenous adenosine

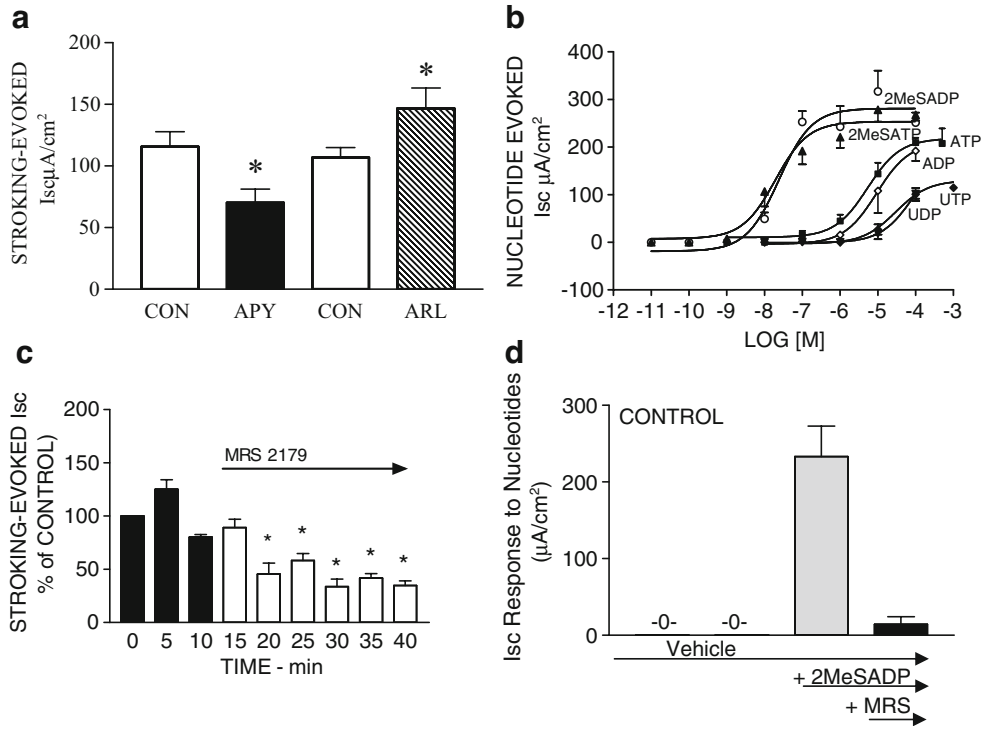


Fig. 7 Mucosal stroking releases endogenous ATP/nucleotides that activate excitatory neural $P2Y_1Rs$ leading to an increase in Isc/chloride secretion in the guinea pig colon. **a** The ATPase/5′-nucleotidase inhibitor 6-*N*,*N*-diethyl-β,γ-dibromomethylene-D-adenosine-5′-triphosphate trisodium (10 μM, ARL 67156 or ARL) prevents hydrolysis of ATP and enhanced reflex-evoked Isc (n=6, *p<0.05), whereas 10 U/ml apyrase hydrolyzes ATP and inhibited reflex-evoked Isc responses (n=4). This indicates that endogenous nucleotide release contributes to the stroking response. **b** Concentration-response curves for nucleotides in the presence of 1 μM CPT (n=4-23), an A_1 receptor antagonist used to block eADO effects. The rank order of potencies of nucleotide agonists for stimulation of Isc was 2MeSADP = 2MeSATP > UTP = UDP that indicates a $P2Y_1R$ interaction. **c** The selective $P2Y_1R$ antagonist MRS2179 suppresses ~50% of the stroking response. **d** The Isc response to the $P2Y_1R$ agonist 2MeSADP was nearly abolished by the $P2Y_1R$ antagonist MRS2179 (10 μM, n=4, p<0.05). All experiments were done in the presence of 1 μM CPT to eliminate inhibitory influence of A_1 receptor activation by endogenous adenosine (n>/4, *p<0.05) (Modified from [27] by permission)

provides a physiological brake to suppress reflex-evoked Cl^- secretion elicited by stroking the mucosa by acting at A_1 receptors located on EC cells [17, 18] and in the ENS [29]. Adenosine acts at A_1 receptors to suppress both the PG and 5-HT sensory limbs of mucosal secretory reflexes [29]. Our understanding of the role of A_1 receptors in the modulation of mucosal reflexes is restricted to the guinea pig colon; however, it is likely this effect will occur in other regions, given that the A_1 receptor provides inhibition of synaptic transmission in myenteric and submucous neurons, and in other regions including stomach antrum, jejunum, and ileum [8, 20, 22].

Distribution of adenosine, P2X, and P2Y receptors and gene products in ENS

Immunohistochemical, gene chip microarray, quantitative reverse transcriptase polymerase chain reaction (RT-PCR), or in situ hybridization data have identified various purinergic receptors in the gut of various species. These include A_1, A_{2a}, A_{2b}, A_3, $P2X_2$, $P2X_3$, $P2X_5$, $P2X_7$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{12}$, and $P2Y_{14}$ receptors in the ENS of guinea pig, mouse, rat, or human intestine [14, 17–19, 25, 27, 29, 49, 93, 94, 108, 109, 117–119].

Differential gene and receptor expression of adenosine A_1, A_{2a}, A_{2b}, and A_3 receptors in the human enteric nervous system

Adenosine A_1, A_{2a}, A_{2b}, and A_3 receptor gene products were differentially expressed in neural and non-neural layers of the human jejunum, ileum, cecum, and colon as well as T-84, HT-29, T98G, and BON/EC cells or in PGP 9.5-ir enteric neurons [19]. Table 1 summarizes adenosine receptor mRNA expression levels in the different layers and cells using RT-PCR. With the exception of the A_1 receptor, mRNA transcripts for A_{2b}, A_{2a}, and A_3 receptors are expressed throughout the GI tract in epithelial cells, mucosa, and submucosa suggesting a significant role in secretomotor behavior of the human gut.

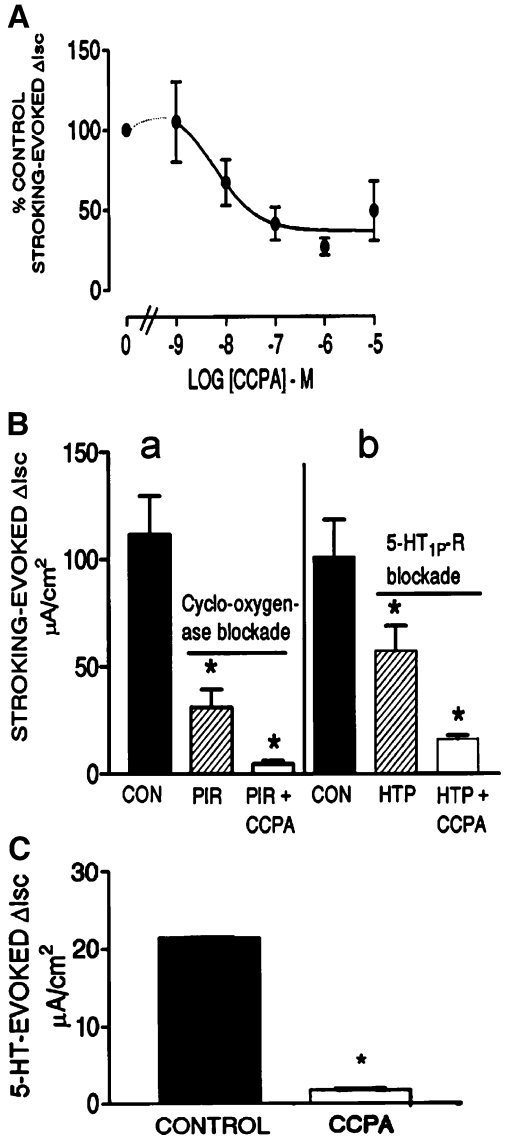


Fig. 8 Effect of CCPA on stroking-evoked change in Isc. **A** Concentration-response curve (EC_{50}=6 nM, n=3-6). **B** Effect of CCPA (0.1 μM) on 5-HT- and PG- mediated limb of reflex pathway. *a* Presence of 5-HT limb after blockade of prostaglandin synthesis with 10 μM piroxicam (PIR) (n=5). *b* Presence of PG limb after blockade of 5-HT limb with 1–10 μM *N*-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (*HTP*) (n=6 or 7). *Con* control, $p<0.05$ from control and PIR or HTP. **C** Effect of CCPA (0.1 μM) on change in Isc evoked by a mucosal pulse of 5-HT (1 μM) (n=3); $p<0.05$ (Modified from [29] by permission)

Cellular localization of adenosine receptor immunoreactivity is summarized in Table 2. Adenosine A_3 immunoreactivity is also expressed in a majority of substance P (~60%) and < 10% of VIP submucous neurons. In contrast to A_3R receptors, A_{2b} immunoreactivity is prominent in 50% of VIP neurons and not expressed in SP neurons in jejunum, but is expressed mainly in non-VIP neurons in colonic submucous neurons. Therefore, A_{2b} receptors and A_3 receptors may serve different roles in secretomotor function in the two regions by acting at putative secretomotor or sensory neurons, respectively.

A_{2a} immunoreactivity is found in a subset of VIP neurons. Varicose fibers in submucous ganglia label for A_{2a} or A_{2b} immunoreactivity. A_{2a} and A_{2b} immunoreactivity is prominent in submucous neurons, but only expressed in a minority of myenteric neurons. Immunoreactivities for A_{2a} and A_{2b} receptors are colocalized in submucous neurons of the jejunum and colon (and jejunal myenteric neurons) as well as in epithelial cells of the jejunum (only region studied). A_{2b} immunoreactivity was also intensely expressed in s-100 positive glia in both nerve plexuses.

A_1 immunoreactivity is abundantly expressed in submucous neurons of the colon, but was undetectable in submucous neurons of the jejunum. This is consistent with functional studies on synaptic Ca^{2+} responses in submucous neurons illustrating a lack of effect of A_1 receptor antagonists

Table 1 RT-PCR to detect human adenosine receptor mRNA in human intestine

	A_1	A_{2a}	A_{2b}	A_3
Jejunum				
Whole thickness	- (-)	- (+)?	++	++
Mucosa/submucosa	- (-)	++	++	+
Mucosa	- (-)	+	++	+
Mucosa	- (+)?	++	++	++
Submucous plexus	- (+)	- (+)	+	+
Longitudinal/circular	+ (++)	++	++	+
Ileum				
Whole thickness	+ (+)	++	+	+
Mucosa/submucosa	ND	ND	ND	ND
Mucosa	- (+)	++	+	+
Submucous plexus	ND	ND	ND	ND
Cecum				
Whole thickness	- (-)	+	++	+
Mucosa/submucosa	- (+)	++	+	+
Mucosa	- (-)	+	++	+
Submucous plexus	- (+)	- (-)	+	- (+)
Colon				
Whole thickness	- (-)	- (-)	+	- (+)?
Mucosa/submucosa	- (-)	- (-)	++	+
Mucosa	ND	ND	ND	ND
Submucous plexus	- (+)	- (+)?	++	+
Human cell lines				
HT-29 (colonic epithelium)	+ (+)	+++	+++	- (-)?
T-84 (colonic epithelium)	- (+)	+	+++	- (-)?
T98G (glioblastoma)	++	+++	+++	- (+)

ND not done, + or – indicate receptor mRNA detected or not detected, respectively. Failure to detect adenosine receptor mRNA after the first round of PCR reflects either absence or low expression. If the results of RT-PCR were negative, an aliquot of the first PCR reaction was amplified a second time, and the result is indicated in parentheses. Failure to detect adenosine receptor mRNA after the second round of PCR is likely to be due to its absence. A *question mark* indicates a possible faint expression of receptor mRNA. (Modified from [19] by permission)

Table 2 Cellular localization of adenosine A_1, A_{2a}, A_{2b}, and A_3 receptor immunoreactivities in human small and large intestine

Human intestinal region/cell type	A_1	A_{2a}	A_{2b}	A_3
Jejunum				+
Longitudinal muscle	±	±	-	
Myenteric plexus neurons	+	+	+	
Glia	-	-	+++	
Circular muscle	+	-	-	
Submucous plexus neurons	-	+++	+++	
Nerve fibers/neurites	-	+	+	+
Epithelia	-	+	+	+
Colon				+
Longitudinal muscle	±	-	-	
Myenteric plexus neurons	-	+	+	
Glia	-	-	+	
Circular muscle	+	+	+	
Submucous plexus neurons	+++	+	+	
Epithelia	+			
T98G				+
U373				+
BON cells				+

- absent, + present (or present ≤ 2 neurons), ± marginally detectable, ++ 3-6 neurons, +++ > 6 neurons. (Modified from [19] by permission)

expected to block ongoing inhibitory effects of endogenous adenosine at neural receptors and enhance synaptic transmission, as revealed in other tissues with functional A_1 receptors (see later discussion; [9, 21]). In addition, strong A_1 immunoreactivity is found in epithelial cells of the human colonic mucosal glands, and mRNA and proteins expressed in two human epithelial cell lines. Therefore, A_1 receptors could potentially play a role in submucosal neural reflexes, leading to suppression of intestinal secretion in the human gut as reported for the guinea pig colon [29]. The diverse but receptor-specific localization and expression of the four adenosine receptors in submucous neurons, glial cells, or epithelial cells, and the colocalization of A_{2a} and A_{2b} receptors in submucous neurons or glandular epithelia, suggests a prominent and complex role for adenosine in the modulation of enteric neural reflexes that control secretomotor functions in the human digestive tract. Functional studies on adenosine receptors in human gut would be of physiological and clinical importance.

Distribution of P2X and P2Y receptors in the enteric nervous system

$P2X_2$ immunoreactivity has been identified in guinea pig ENS [14, 50, 111]. In submucous ganglia of the guinea pig ileum, $P2X_2$ immunoreactivity occurred in all VIP-positive neurons and calbindin-positive neurons making up about 50% of the neurons. In contrast, there was not any $P2X_2$ immunoreactivity in NPY or calretinin-positive neurons. Overall, $P2X_2R$ are expressed in both nerve plexuses, in inhibitory motor neurons, IPANs of the myenteric plexus in stomach and intestine, on gastric endings of vagal afferent fibers, VIP noncholinergic secretomotor neurons, and IPANs of the submucous plexus [14]. In contrast to $P2X_2$, $P2X_3$ immunoreactivity in the submucous plexus occurs in most calretinin neurons that represent cholinergic neurons projecting to mucosa or arterioles, and make up ~12% of the population [10]; therefore, the activity of some secretomotor neurons is likely to be modulated by $P2X_3$ receptors in guinea pig.

$P2X_2$ and $P2X_3$ immunoreactivity was also identified in nerve fibers in enteric ganglia, interganglionic fiber tracts, in the muscularis as well as in the perivascular plexus of the *guinea pig gallbladder*. $P2X_2$ or $P2X_3$ neurons displayed immunoreactivity for VIP (>90%) and NOS (~50-60%) [93]. The function of these receptors remains unknown.

$P2X_2$ and $P2X_3$ immunoreactivity was also localized in rat ENS, by in situ hybridization or mRNA levels [119]. Receptors were identified throughout the GI tract in both myenteric and submucous neurons. $P2X_2$ immunoreactivity (56% ileum, 45% distal colon) and $P2X_3$ immunoreactivity (62%, 40%) was abundant in submucous neurons. $P2X_2$ immunoreactivity in submucous plexus colabeled for calbindin or calretinin in 30-50% of neurons. $P2X_3$ immunoreactivity neurons expressed calretinin (40%) and calbindin (30-75%). Coexpression of $P2X_3$ immunoreactivity in calbindin neurons also occurred in colorectum [116] but not in those in rat ileum [85, 108]. At least in the mouse, AH sensory (not S/type 1) neurons did express $P2X_3$ immunoreactivity [7]. Species and region differences may exist in distribution of the $P2X_2$ and $P2X_3$ receptors in submucous neurons (see [7, 14, 49, 93, 111, 118]). The $P2X_1$, $P2X_4$, or $P2X_6$ receptor was undetectable in mouse ENS. The $P2X_5$ receptor is widely distributed in mouse GI tract in enteric ganglia in both nerve plexuses. In the submucous plexus, $P2X_5$ immunoreactivity discretely colocalize with 22% of calretinin, 9% of calbindin, 6% of nitric oxide synthase, and 68% of VIP-positive neurons. Therefore, the $P2X_5$ receptor may be distributed in secretomotor and intrinsic sensory neurons. The mouse $P2X_5$ receptor could form heteromultimers with a unique pharmacology [94].

$P2X_3$ immunoreactivity has also been identified in submucous (and myenteric) neurons as well as in some axons/dendrites of the *human colon* [36, 122], but the identity of the subtypes of human neurons expressing $P2X_3$ immunoreactivity remains unclear. Future studies should include detailed analysis of the distribution of purinergic receptors in the human ENS.

$P2Y_2$ immunoreactivity occurs in neurons and fibers in both myenteric and submucous plexuses in the corpus of

the stomach, jejunum, ileum, and colon of the guinea pig [118]. $P2Y_2$ immunoreactivity is prominent in neurons with Dogiel type I morphology. All $P2X_3$ neurons and none of the $P2X_2$ neurons in the submucous plexus (or ~50% in myenteric neurons) coexpressed $P2Y_2$ immunoreactivity throughout the GI tract. A majority of calretinin and NPY submucous neurons also expressed $P2Y_2$ immunoreactivity. Submucosal neurons with $P2X_3$ and $P2Y_2$ receptor immunoreactivity with Dogiel type I morphology may represent a subset of S/type 1 neurons with Dogiel type I morphology that display both a fast P2X and slow P2Y membrane depolarization [3]. It has been suggested that a $P2X_2$ or $P2X_7$ receptors may mediate the fast P2X response in neurons that only display a fast response [3, 14, 49, 119]. Lack of $P2Y_2$ immunoreactivity in calbindin submucosal neurons of the guinea pig gut argues against their expression on IPANs [39]. Regional differences occur in the colocalization of $P2Y_2$ immunoreactivity with $P2X_3$ immunoreactivity or in other types of neuronal markers. These receptors may play a role in regulation of mucosal secretory glands and the local vasculature.

The $P2Y_6$ receptor is widely distributed in myenteric but not submucous ganglia of the stomach, jejunum, ileum, and colon (in guinea pig), whereas the $P2Y_{12}$ receptor is also widely distributed in submucous ganglia. $P2Y_{12}$ and $P2Y_6$ receptors and neurons likely play different roles in the gut. A distinction between their localization is that $P2Y_{12}$ immunoreactivity (not $P2Y_6$) is also expressed in calbindin intrinsic sensory neurons and not calretinin or NOS (in both plexuses). $P2Y_6$ immunoreactivity is instead found in NOS and calretinin-positive neurons. Therefore, the $P2Y_{12}$ receptor may play a role in sensory signaling in mucosal secretomotor reflexes.

Mucosal epithelial cell lines and ion transport

In human epithelial carcinoma cell lines (HCT8 and Caco-2), cells express mRNA for $P2X_1$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, and $P2X_7$ and $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, and $P2Y_{12}$ receptors. In addition, $P2Y_1$, $P2Y_2$, and $P2X_{1\text{-}7}$ receptor proteins are expressed in these cells. Functional studies on cell proliferation and apoptosis using selective agonists and antagonists suggested involvement of $P2X_7$, $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors but their role in secretion awaits further study [31].

ENaC and CFTR channels

ENaC mediate electrogenic absorption of Na^+ in intestine (and other epithelial tissues). ENaC in colonic epithelia is expressed together with the CFTR Cl^- channels. Stimulation of purinergic receptors by ATP or activation of CFTR stimulate chloride secretion and inhibit amiloride-sensitive Na^+ transport (ENaC). Inhibition of ENaC by nucleotides is not via CFTR-mediated ATP release [62]. ATP or UTP acting at P2YRs also activate Ca^{2+}-dependent Cl^- channels. Na^+ absorption in mouse colon (and airway epithelia) is inhibited by cell shrinkage (change in volume) by a mechanism that does not interfere with purinergic (or CFTR) mediated inhibition of ENaC [101]. A recent study in $P2Y_2$ and $P2Y_4$ knockout mice suggests that only the stimulation of the luminal $P2Y_2$ receptor mediates inhibition of electrogenic Na^+ absorption via epithelial Na^+ channels (ENaC) in mouse colon. In contrast, luminal $P2Y_2$ and $P2Y_4$ receptors stimulate K^+ secretion [74].

Luminal P2 and ion transport

P2 receptors are found in both the basolateral and luminal membranes. A recent review by Leipziger (2003) summarizes the different P2X and P2Y receptors, their functions, and intracellular signaling pathways in all epithelial tissues.

Relevant information on gut luminal P2 receptors is summarized in Table 3. Several generalizations can be made from Table 3. Epithelial cells respond well to ATP and UTP. $P2Y_2$ and $P2Y_4$ receptors are very prevalent luminal receptors involved in Cl^-, HCO_3^-, K^+ secretion, or mucin secretion. Nucleotide stimulation of K^+ secretion occurs in the distal colon, K^+ secretion and HCO_3^- secretion in the gallbladder (Table 3; [17, 18, 75, 90]); HCO_3^- secretion also occurs in ducts and is involved in the formation of pancreatic juice. Stimulation of Cl^- secretion occurs in the gallbladder, small intestine, and duct cells. Ca^{2+} is the main second messenger coupled to P2Y receptors, although cAMP can be involved as well. Region and species differences occur especially in $P2X_7$ and $P2Y_6$ receptors. Functional discrimination between $P2Y_2$ and $P2Y_4$ receptors is difficult because of the similar agonist potency profiles [102]. The persistence of a Cl^- secretory response to UTP or ATP in $P2Y_2^{-/-}$ mice suggested that the response *in mice* could be mediated by $P2Y_4$ receptors [32]. Indeed, loss of nucleotide regulation of epithelial chloride transport occurs in the jejunum of $P2Y_4$-null mice [90]. $P2Y_4$ mRNA are expressed in rat and guinea pig submucosa [17, 18, 27], murine colonic crypts [75], stomach and intestine [90, 105], and more recently in enteric glial cells [108, 109]. In the guinea pig small intestine, $P2Y_4$ immunoreactivity is expressed in enteric glial cells (EGCs) in various regions of the GI tract except esophagus. EGCs are important regulators of barrier function, mucosal permeability, neuronal activity, and epithelial cell growth and are active participants in intestinal inflammation [13, 95]. Some discrepancy exists between studies on whether $P2Y_4$

Table 3 Functional distribution of luminal P2 receptors in GI epithelial cells

Tissue	Species	P2 receptor	Endog. agonist	Function	Signaling	Reference
Jejunum	Mouse	$P2Y_4$	ATP = UTP	Cl^- secretion↑		32
Duod. villus	Rat	$P2X_7$		Apoptosis ??		43
Pancr. duct	Guinea pig	$P2Y_2$	ATP = UTP	HCO_3^- secretion↑	Ca^{2+}↑	52
	Rat	$P2X_7$	Bz-ATP		Ca^{2+}↑	47
	Rat	$P2Y_2$, $P2Y_4$, $P2X_7$	UTP/ATP	Cl^- secretion↑	Ca^{2+}↑	72
	Dog		ATP	Mucin secret. ↑	Ca^{2+}↑	81
PDEC	Human	$P2Y_2$/$P2Y_4$	UTP/ATP	Cl^- secretion↑	Ca^{2+}↑	16
CFPAC-1	Mouse	$P2Y_2$/$P2Y_6$	ATP = UTP/UDP	Cl^- secretion↑		32
Gallbladder	Mouse	$P2Y_2$	ATP/UTP	HCO_3^-secret.↑	Ca^{2+}↑	23
	Necturus	P2	ATP	Cl^-secretion↑	cAMP↑	107
Bile duct	Rat	$P2Y_1$, $P2Y_2$	ATP, UTP	HCO_3^-secret.↑		
		$P2Y_4$, $P2Y_6$	ADP, UDP			
			2MeSATP			
	Rat	$P2Y_2$/$P2Y_4$	UTP/ATP	Cl^- secretion↑		96, 100
Colon	Rat, mouse	$P2Y_2$/$P2Y_4$	UTP/ATP	K^+ secretion↑	Ca^{2+}↑	56
				Na^+ absorption↓	?	
Caco-2	Human	$P2Y_2$, $P2Y_4$, $P2Y_6$	UTP/ATP	Cl^- secretion↑	Ca^{2+}↑	76

immunoreactivity occurs in epithelial cells, but EGCs may play a role in the modulation of ion transport in gut epithelial cells (for review see [95]).

Little evidence exists that luminal P2 receptors activate absorption, other than the preposition that a $P2X_7$ receptor is involved in stimulation of Na^+/H^+ exchanger type 3-mediated Na^+ absorption in rat submandibular gland ducts [66]. In contrast, data suggest that luminal fluid secretion and a diarrhea response would occur with activation of luminal P2Y receptors in small intestine (Cl^- secretion) or colon (inhibition of Na^+ absorption via ENaCs and K^+ secretion); a diarrhea response may play a role in host defense reactions against potential pathogenic invading organisms. Another possibility is that the $P2X_7$ receptor in villus tip cells that undergo programmed cell death and exfoliation may act as a "death signal" to insure villus cell regeneration [43]. The $P2Y_6$ receptor is a potential therapeutic target in the treatment of cystic fibrosis gallbladder disease because UDP retained its ability to promote/stimulate Isc/ion transport changes in cystic fibrosis gallbladder epithelial cells from CFTR-deficient mice [65].

Basolateral $P2Y_6$ stimulates NaCl secretion

Recent evidence suggests that *basolateral* $P2Y_6$ receptors that are sensitive to UDP in colonic epithelia cells stimulate sustained NaCl secretion by evoking a synergistic increase in intracellular free Ca^{2+} and cAMP. In *Xenopus* oocytes coexpressing $P2Y_6$ with the cystic fibrosis transmembrane conductance regulator (CFTR), UDP transiently activated the Ca^{2+}-activated Cl^- current and subsequently CFTR.

A_{2b} receptors in apical secretion

Adenosine is generated at sites of tissue injury or stress including ischemia, inflammation, and tissue remodeling by ATP catabolism. Adenosine at inflammatory sites can have either proinflammatory or anti-inflammatory effects depending on the receptor or tissue. In crypt abscesses during periods of active inflammation, ectonucleotidases convert ATP derived from neutrophils to adenosine. Adenosine causes vectorial chloride and interleukin-6 secretion [33, 73, 103]. In human jejunum, an A_2 receptor agonist 5′-*N*-methylcarboxamidoadenosine stimulates chloride secretion. A_{2b} immunoreactivity is prominent in human mucosa or epithelial cells of the cecum, colon, and jejunum (Christofi et al. 1999; [89, 104]).

At rest, in epithelial cells, the A_{2b} receptor is intracellular and inactive, and agonist stimulation on the apical or basolateral side of human epithelial cells induces polarized trafficking and surface expression of the A_{2b} receptor in intestinal epithelial cells [33]. *N*-ethylmaleimide attachment receptor (SNARE) proteins participate in recruitment of A_{2b} receptors that may be necessary for its signaling. The A_{2b} receptor signals through the cAMP/protein kinase A/cAMP response-element binding protein to cause apical chloride and interleukin-6 secretion. It has been suggested that interleukin-6 would promote neutrophil degranulation

and enhance microbicidal activity of neutrophil trafficking to the intestinal mucosa [103].

Purinergic regulation of secretion from neuroendocrine cells in gastric mucosa

A1/gastrin release In the stomach, adenosine protects against stress-induced gastric ulcer formation by inhibiting gastric acid secretion by direct action on parietal cells in some species (guinea pigs and dogs, [42, 48]) but not others (rats, [86]). A recent study using selective A_1 (*N*6-cyclopentyladenosine), A_{2a} (CGS 2168), and A_3 (IB-MECA) receptor agonists provided pharmacological proof that an A_1 receptor is negatively coupled to gastrin release in rat stomach. An A_1 receptor selective antagonist 8-cyclopentyl-1,3-dipropyl-xanthine abolished adenosine effects on gastrin release, and A_1 immunoreactivity was found on mucosal G cells (gastrin) and D cells (somatostatin), but not parietal cells (H^+ ions, acid). Therefore, adenosine may suppress gastrin release by activating A_1 receptors on G cells, leading to inhibition of gastric acid secretion [123, 125].

Omeprazole-induced achlorhydria suppresses gene expression of both A_1 and A_{2a} receptors in the antrum and corpus mucosa. In the vascularly perfused rat stomach, omeprazole also significantly attenuated the A_{2a} receptor-mediated stimulation of somatostatin release along with a corresponding decrease in A_{2a} receptor mRNA expression. This acid-dependent change in A_{2a} receptor expression is a regulatory feedback mechanism to control gastric acid secretion [124]. The A_{2a} receptor agonist ATL-146e could almost prevent aspirin-induced gastric mucosal lesions [83].

Clinical implications of adenosine deaminase (ADA) activity Clinical studies suggest that adenosine inhibits gastric acid secretion and may act as a gastroprotective agent. For instance, patients suffering from acid hypersecretion have higher ADA activity. There is a direct correlation between ADA activity and gastric acid output in fundic mucosa of patients with ulcer, gastritis, and achlorhydria [79]. In patients with gastric ulcers, corpus mucosa ADA activity is reduced after H2 blocker ranitidine treatment [80].

A_{2a} receptors and somatostatin release Adenosine administration to the isolated vascularly perfused rat stomach inhibits gastrin release and stimulates somatostatin release [64], a potent inhibitor of gastric acid secretion. Adenosine analogs augment somatostatin-like immunoreactivity release with a potency profile of CGS 21680= 5′-*N*-ethylcarboxamidoadenosine > 2-chloroadenosine > R-(-)-*N*6-(2-phenylisopropyl)adenosine >1-deoxy-1-[6-[[3-iodophenyl)methyl]amino]-9H-purin-9-yl]-*N*-methyl-β-D-ribofuranuronamide > *N*6-cyclopentyladenosine = *N*6-cyclohexyladenosine > *S*-(+)-*N*6-(2-phenylisopropyl) adenosine, suggesting an A_{2a} receptor involvement. The A_{2a} receptor antagonist ZM 241385 abolished the CGS 21680 or adenosine stimulation of somatostatin release. Immunochemical distribution of A_{2a} receptors suggests that adenosine can act on D cells or indirectly on myenteric neurons to stimulate somatostatin release and consequently suppress gastric acid secretion [125].

Gastric preconditioning induced by short ischemia (brief occlusion of celiac artery for 5 min) protects against mucosal damage induced by severe ischemia/reperfusion or topical mucosal irritants in the stomach. Adenosine release among other mediators [63] contributes to protection. Adenosine pretreatment (10 mg/kg i.g.) reduced lesions and improved gastric blood flow similar to ischemic preconditioning. The adenosine receptor antagonist 8-phenyltheophiline (10 mg/kg i.g.) attenuated the gastroprotection due to preconditioning. Furthermore, the selective A_{2a} receptor agonist ATL-146e (5 μg/kg) attenuated stress-induced gastric lesions and damage and reduced production of proinflammatory cytokines [tumor necrosis factor (TNF)-α, interleukin (IL)-1β] and neutrophil infiltration, suggesting a protective effect via A_2 receptors against ulcer formation [82].

Reflex-driven intestinal diarrhea and clinical relevance of purines

The therapeutic potential of purinergic receptors in intestinal secretomotor disorders remains to be established, but evidence is building in support of endogenous adenosine, A_{2a}, and A_3 receptors as therapeutic targets. Toxin A (TxA) release from *Clostridium difficile* (a gram-positive anaerobic bacillus) can contribute to antibiotic-induced diarrhea and pseudomembranous colitis; the latter condition is associated with significant mucosal secretion and inflammation. The neural circuit mechanism deduced from the available evidence is shown in Fig. 9. Briefly, activation of nuclear factor NF-κB initiates a cascade of events involving epithelial cells, macrophages, neutrophils, mast cells, extrinsic primary afferent neurons (EPANs), and the ENS including cholinergic secretomotor neurons. Eventual hyperexcitability of neural circuits leads to amplification of the secretory response and profuse secretion to flush out the pathogenic organism [25]. The selective A_{2a} receptor agonist ATL 313 (0.5-5 nM) also reduced *Clostridium difficile* TxA-induced secretion and edema, prevented the mucosal damage and neutrophil infiltration, TxA-induced

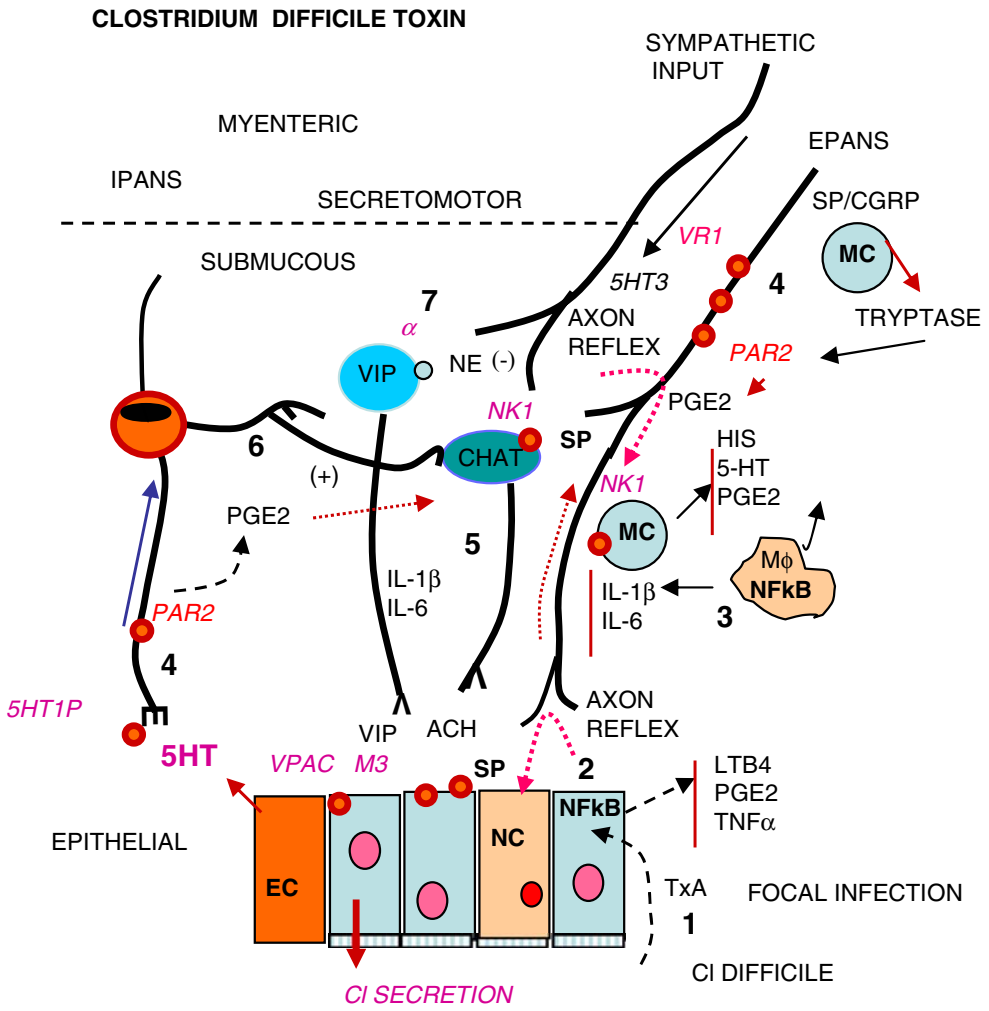


Fig. 9 *1 Clostridium difficile* toxin A (*TxA*) stimulates reflex-evoked intestinal secretion and causes diarrhea. *2* After TxA is internalized in enterocytes, TxA activates NF-kB and releases LTB4, PGE2, TNF. *3* After NF-kB activation, the proinflammatory mediators interleukins (*IL*) are released from monocytes/macrophages (*MO*). *4* Stimulation of processes of EPANS occurs, followed by release of substance P (*SP*)/CGRP that degranulates mast cells (*MC*) by activating NK1 receptors. *5* SP-containing EPANS release SP on cholinergic secretomotor neurons. The mediators released from mast cells include histamine, 5-HT, PGE2, and tryptase. *6* Tryptase acts on PAR2 receptors on EPANS and on IPANS to stimulate further release of PGE2. *7* The sympathetic innervation with norepinephrine release and its binding to α2-adrenergic receptors normally reduces secretion via VIP secretomotor neurons. ILs suppress norepinephrine release. These cytokines act on sympathetics to lift the sympathetic brake exerted by norepinephrine. TxA-induced release of proinflammatory mediators degranulates mast cells that amplify neural and secretory responses in order to maximize secretion in defense of the host. Treatment with an A_{2a} receptor agonist attenuates the TxA-induced diarrhea presumably by acting in this reflex pathway. (Modified from [25] by permission)

cell death, TNF-α production, as well as increased ileal ADA activity, in murine ileal loops exposed to TxA. Effects were reversed by the selective A_{2a} receptor antagonist ZM241385 (5 nM) [15].

Recent studies provided convincing evidence that activation of an A_{2a} receptor attenuates intestinal inflammation in animal models of IBD and may serve as a novel therapy for IBD [15, 77, 82]. In general, adenosine has diverse functions in the GI tract in the regulation of secretion, motility, innate mucosal immunity, is protective against experimental IBD, and is important in mediating inflammatory responses at sites of injury, and has anti-inflammatory effects in epithelial cells.

Adenosine is also reported to be a conditionally essential nutrient for the gut and has been shown to be beneficial in alleviating severe diarrhea in children with cholera [20, 25, 98].

Recent findings indicate that lumenal adenosine or AMP can rapidly increase glucose transport by small intestine and increase systemic availability of 3-*O*-methyl glucose after an oral administration to mice. The putative A_2 receptor involved is linked to a signaling pathway involving cAMP production. Adenosine causes a rapid increase in carrier-mediated glucose uptake that is suggested to be of clinical relevance [60]. In contrast to other modulators, adenosine is a natural component of the diet, and this alone or as

supplement in the diet may suggest potential applications in treatment of malabsorption in chronic intestinal inflammatory diseases. Daily intake of purines is 600-1,000 mg/day by adult individuals in the USA [99]. It has been estimated that diet and endogenous adenosine production can contribute 5-6 mM of adenosine in the lumen in individuals on a Western diet. A concentration of 1 mM luminal adenosine caused a maximum effect on glucose uptake [60].

In human intestinal epithelial cells, adenosine affects immune function and exerts anti-inflammatory effects by acting as a negative regulator of NF-κB and MAPK signaling. Adenosine pretreatment in HT-29 cells caused a reduction in stimulated IL-8 expression and secretion [55].

Neurogenic diarrhea

The adenosine A_3 receptor is one of the least understood receptors in secretomotor reflexes. The physiological relevance of A_3 receptor is unclear, and current opinion is that it may function primarily in disease or abnormal states such as ischemia/reperfusion injury, tissue damage, gut inflammation, and extremes in motor activity of the gut when endogenous adenosine release is sufficiently high to activate the low affinity receptor [53]. It has been reported by Cooke et al. [30] that dimaprit–H_2R activation of submucous neurons leads to a stereotypical cyclical pattern of secretion in coordination with motility that can last for several hours. The underlying electrophysiological correlate is cyclical depolarization and excitation of submucous neurons. This is a model of neurogenic diarrhea involving an immune-neural circuit. It proved useful in elucidating a functional role of A_3 receptors in addition to A_1 receptors in the secretomotor function and coordination of motility and secretion [8, 9]. Dimaprit was used to elicit a stereotypical cyclical increase in short-circuit current (Isc = chloride secretion) in coordination with motility. Isc was recorded in guinea pig distal colon simultaneously with muscle length in the circular orientation by sonomicrometry. A_1 receptor-selective antagonists 8-cyclopentyltheophylline (CPT) or 1,3-dipropyl-8-(2-amino-4-chlorophenyl) xanthine (PACPX) or the irreversible antagonist FSCPX caused a concentration-dependent augmentation of coordinated responses; 100 nM antagonist caused maximum blockade of A_1 receptors and enhanced Isc 133% and ICD 64% with ED_{50} values in the nanomolar concentration range. Dimaprit responses were abolished by 10 nM A_1 receptor agonist CCPA and reversed by A_1 receptor antagonists. After knockdown of A_1 receptors with FSCPX (1 μM), the selective A_3 receptor agonist IB-MECA could still abolish coordinated responses (IC_{50}-Isc=2.1 μM; IC_{50}-ICD=7.1 μM) that could be reversed by the A_3 receptor antagonist MRS1191. The A_3 receptor antagonist alone could also enhance Isc and coordinated muscle responses. The coordinated response to dimaprit is abolished by nerve blockade with TTX, and remaining myogenic activity was reduced by ~50% by IB-MECA after A_1 receptor knockdown with FSCPX. MRS1191 (1 μM) could enhance TTX-sensitive distension-evoked Isc responses. A_3 receptor immunoreactivity occurs in myenteric and submucous neurons of guinea pig, mouse, rat, and human colon (Christofi, unpublished observations). Availability of knockout models for adenosine receptor subtypes should provide unequivocal proof of function.

In summary, preliminary data suggest that in a model of neurogenic diarrhea endogenous adenosine release is sufficient to act at inhibitory A_1 and A_3 receptors to modulate the stereotype neural-motor behavior triggered by the mast cell mediator histamine. Adenosine A_3 receptors may be targets for modulation of distension-evoked neurosecretory reflexes as well. The role of A_3 receptors in bowel disorders associated with diarrhea or diarrhea predominant IBS warrants further investigation.

Differential dysregulation of purinoceptors in GI disease states

As already described, purinergic signaling regulates all important physiological functions of the GI tract including epithelial transport, 5-HT release from EC cells, mucosal reflexes, sensory signaling in the ENS, synaptic transmission, as well as coordination of motility and secretion. It is reasonable then to suggest that these abnormalities in purinergic receptors or signaling pathways would have a significant impact on the overall behavior of the gut, enteric reflexes, and secretomotor behavior of the gut in bowel diseases. Purinergic receptors in gastrointestinal inflammation were the subject of a recent review by Kolachala et al. [61]. A recent study by Guzman et al. [45] using high-density oligonucleotide microarray analysis and SYBR-Green RT-PCR validation provided direct proof for differential dysregulation (upregulation or downregulation depending on the purine gene) of $P2X_1$, $P2X_2$, $P2X_4$, $P2X_7$, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, A_{2a}, A_{2b}, A_1, or A_3 receptors in whole thickness gut specimens of experimental trinitrobenzene sulfonic acid (TNBS) colitis in rats . Expression of A_1 and A_3 receptor gene products is also altered in a chronic ileitis model of Crohn's disease [106]. The ATP-gated ion channel $P2X_3$ is increased in human IBD [122] as is the case for TNBS colitis [45]. In submucosal neurons of TNBS colitis, there is emergence of a purinergic component of the fast EPSP that is cholinergic in nature in normal animals [69, 70]. Therefore, there is plasticity of purinergic neurotransmission. Abnormalities in the neural regulation of the gastrointestinal vasculature could contribute to the pathogenesis of IBD. Recent data suggest that reduced

purinergic neurotransmission via putative $P2X_1$ receptors may underlie a defect in sympathetic regulation of gut vasomotor function during TNBS colitis [68]. Changes in vasomotor function could also impact on secretomotor responses. Further studies are needed to identify the location of these receptors in the gut wall and better understand the functional relevance of these and other receptor abnormalities on secretomotor and vasomotor function in various species and in human gut.

It was recently shown that purinergic gene dysregulation in experimental colitis is sensitive to oral administration of the A_3 receptor agonist IB-MECA, a potential therapeutic target for IBD [45]. IB-MECA protects against tissue injury, development of colitis, and prevents dysregulation of 92% of genes in TNBS colitis, including purine genes. This illustrates a new important benefit of A_3 receptor agonists in preventing abnormalities in purine gene expression in colitis. A review by Antonioli et al. [2] discusses the pharmacological modulation of adenosinergic pathways in the therapeutic management of IBD. Adenosine A_1, A_{2a}, A_{2b}, and A_3 receptors are potential therapeutic targets as regulators of gut enteric immune responses and all components of gut motor reflexes [2, 27].

Summary and conclusions

A secretomotor reflex is activated by mucosal stroking, distension, or chemical stimulation of the mucosa leading to release of 5-HT or other mediators, subsequent activation of IPANs in synaptic communication with interneurons or secretomotor neurons to stimulate chloride and fluid secretion. Inputs from myenteric neurons modulate secretory rates and reflexes, and special neural circuits exist to coordinate secretion with motility and vasomotor functions. All cellular components of secretomotor reflexes express multiple purinergic receptors for adenosine or the nucleotides ATP, ADP, UTP, or UDP. This review focused on the emerging concepts in our understanding of purinergic regulation at these receptors, and in particular of mechanosensory reflexes. Endogenous purines can activate inhibitory A_1, A_3, and $P2Y_{12}$ receptors or stimulatory A_2 and $P2Y_1$ receptors to modulate 5-HT release. The resting "secretory tone" is influenced by endogenous purines. Stimulatory $P2Y_1$ receptor or inhibitory A_1/A_3 receptors are involved in mechanically evoked secretomotor reflexes and a model of neurogenic diarrhea. Recent studies identified the neural and/or non-neural distribution profiles of $P2X_2$, $P2X_3$, $P2X_5$, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, or $P2Y_{12}$ receptor immunoreactivity in the GI tract suggesting their role in secretomotor function. Significant species and regional differences exist in distribution and function of the receptors. Abnormal expression of purinergic receptors may contribute to functional abnormalities and diarrhea in IBD; functional studies are lacking. Adenosine A_{2a} or A_3 receptors are emerging as therapeutic targets in IBD and protect against purinergic receptor abnormalities or profuse diarrhea. Purines are emerging as fundamental regulators of enteric secretomotor reflexes in health and disease.

Future studies should be aimed at better understanding the function of other purinergic receptors expressed in the enteric nervous system or non-neuronal components of the reflex, in both rodents and human gut. More integrative studies on purinergic regulation of chemosensory or mechanosensory secretomotor reflexes are desperately needed. This should pave the way to better understand purine receptor biology, abnormalities in disease states, or as therapeutic targets.

Acknowledgements Many thanks to Drs. Helen J. Cooke, Jacqueline Wunderlich, Jun Ge Yu, Shelly Xue, Jorge Guzman, Huiming Zheng, Asad Javed, and Iveta Grants for their research contributions to the overall field of study. Some cited studies and this review were supported by the National Institutes of Health (NIH) grant numbers R01 DK44179 years 1–13, a 3 year Fellowship to Dr Jorge Guzman and NCRR 1S10RR11434 and OSU-seed funds.

References

1. Abbracchio MP, Burnstock G, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Knight GE, Fumagalli M, Gachet C, Jacobson KA, Weisman GA (2006) International Union of Pharmacology LVIII: update on the P2Y G protein-coupled nucleotide receptors: from molecular mechanisms and pathophysiology to therapy. Pharmacol Rev 58(3):281–341
2. Antonioli L, Fornai M, Colucci R, Ghisu N, Tuccori M, Del Tacca M, Blandizzi C (2008) Pharmacological modulation of adenosine system: novel options for treatment of inflammatory bowel diseases. Inflamm Bowel Dis 14:566–574
3. Barajas-Lopez C, Espinosa-Luna R, Christofi FL (2000) Changes in intracellular Ca^{2+} by activation of P2 receptors in submucosal neurons in short-term cultures. Eur J Pharmacol 409 (3):243–257
4. Bearcroft CP, Perrett D, Farthing MJ (1998) Postprandial plasma 5-hydroxytryptamine in diarrhoea predominant irritable bowel syndrome: a pilot study. Gut 42(1):42–46
5. Bertrand PP (2006) Real-time measurement of serotonin release and motility in guinea pig ileum. J Physiol 577(Pt 2):689–704
6. Bertrand PP (2003) ATP and sensory transduction in the enteric nervous system. Neuroscientist 9(4):243–260
7. Bian X, Ren J, DeVries M, Schnegelsberg B, Cockayne DA, Ford AP, Galligan JJ (2003) Peristalsis is impaired in the small intestine of mice lacking the P2X3 subunit. J Physiol 551(Pt 1):309–322
8. Boserov A, Wang YZ, Javed N, Christofi FL, Cooke HJ (2005) Comparison of adenosine A1 receptor activation on repetitive reflex Cl secretion coordinated with repetitive muscle activity measured by sonomicrometry and force transducer tension. Gastroenterology 128(4 Suppl 2):A–608
9. Bozarov A, Wang Y-Z, Yu JG, Cooke HJ, Christofi FL (2006) Endogenous adenosine acts at A1 or A3 inhibitory receptors to modulate the stereotype neural-motor behavior of the gut triggered by histamine or distension. Gastroenterology 130(4 Suppl 2):A–29

10. Brookes SJ, Steele PA, Costa M (1991) Identification and immunohistochemistry of cholinergic and non-cholinergic circular muscle motor neurons in the guinea-pig small intestine. Neuroscience 42(3):863–878
11. Brookes SJ (2001) Classes of enteric nerve cells in the guinea-pig small intestine. Anat Rec 262(1):58–70
12. Burnstock G (2006) Purinergic signalling. Br J Pharmacol 147 (Suppl 1):S172–S181
13. Cabarrocas J, Savidge TC, Liblau RS (2003) Role of enteric glial cells in inflammatory bowel disease. Glia 41(1):81–93
14. Castelucci P, Robbins HL, Poole DP, Furness JB (2002) The distribution of purine P2X(2) receptors in the guinea-pig enteric nervous system. Histochem Cell Biol 117(5):415–422
15. Cavalcante IC, Castro MV, Barreto AR, Sullivan GW, Vale M, Almeida PR, Linden J, Rieger JM, Cunha FQ, Guerrant RL, Ribeiro RA, Brito GA (2006) Effect of novel A2A adenosine receptor agonist ATL 313 on Clostridium difficile toxin A-induced murine ileal enteritis. Infect Immun 74(5):2606–2612
16. Chan HC, Cheung WT, Leung PY, Wu LJ, Chew SB, Ko WH, Wong PY (1996) Purinergic regulation of anion secretion by cystic fibrosis pancreatic duct cells. Am J Physiol 271(2 Pt 1):C469–C477
17. Christofi FL, Kim M, Wunderlich JE, Xue J, Suntres Z, Cardounel AJ, Javed NH, Yu JG, Grants I, Cooke HJ (2004) Endogenous adenosine differentially modulates 5-hydroxytryptamine from a human enterochromaffin cell model. Gastroenterology 127:188–202
18. Christofi FL, Wunderlich J, Yu JG, Wang YZ, Xue J, Guzman J, Javed N, Cooke H (2004) Mechanically evoked reflex electrogenic chloride secretion in rat distal colon is triggered by endogenous nucleotides acting at P2Y1, P2Y2, and P2Y4 receptors. J Comp Neurol 469(1):16–36
19. Christofi FL, Zhang H, Yu JG, Guzman J, Xue J, Kim M, Wang YZ, Cooke HJ (2001) Differential gene expression of adenosine A1, A2a, A2b, and A3 receptors in the human enteric nervous system. J Comp Neurol 439(1):46–64
20. Christofi FL (2001) Unlocking mysteries of gut sensory transmission: is adenosine the key? News Physiol Sci 16:201–207
21. Christofi FL, Wood JD (1993) Presynaptic inhibition by adenosine A1 receptors on guinea pig small intestinal myenteric neurons. Gastroenterology 104(5):1420–1429
22. Christofi FL, Tack J, Wood JD (1992) Suppression of nicotinic synaptic transmission by adenosine in myenteric ganglia of the guinea-pig gastric antrum. Eur J Pharmacol 216(1):17–22
23. Clarke LL, Harline MC, Gawenis LR, Walker NM, Turner JT, Weisman GA (2000) Extracellular UTP stimulates electrogenic bicarbonate secretion across CFTR knockout gallbladder epithelium. Am J Physiol Gastrointest Liver Physiol 279(1):G132–G138
24. Coates MD, Mahoney CR, Linden DR, Sampson JE, Chen J, Blaszyk H, Crowell MD, Sharkey KA, Gershon MD, Mawe GM, Moses PL (2004) Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 126(7):1657–1664
25. Cooke HJ, Christofi FL (2006) Enteric neural regulation of mucosal secretion. In: Johnson LR (ed) Physiology of the gastrointestinal tract, 4th edn. Academic, New York, pp 737–762
26. Cooke HJ, Wunderlich J, Christofi FL (2003) "The force be with you": ATP in gut mechanosensory transduction. News Physiol Sci 18:43–49
27. Cooke HJ, Xue J, Yu JG, Wunderlich J, Wang YZ, Guzman J, Javed N, Christofi FL (2004) Mechanical stimulation releases nucleotides that activate P2Y1 receptors to trigger neural reflex chloride secretion in guinea pig distal colon. J Comp Neurol 469(1):1–15
28. Cooke HJ, Javed N, Christofi FL (2003) Enteric neural reflexes and secretion. In: Bienenstock J, Goetzl EJ, Blennerhassett MG (eds) Autonomic neuroimmunology, 1st edn. CRC Press, Boca Raton, pp 35–59
29. Cooke HJ, Wang Y, Liu CY, Zhang H, Christofi FL (1999) Activation of neuronal adenosine A1 receptors suppresses secretory reflexes in the guinea pig colon. Am J Physiol 276(2 Pt 1):G451–G462
30. Cooke HJ, Wang YZ, Rogers R (1993) Coordination of Cl-secretion and contraction by a histamine H2-receptor agonist in guinea pig distal colon. Am J Physiol 265(5 Pt 1):G973–G978
31. Coutinho-Silva R, Stahl L, Cheung KK, de Campos NE, de Oliveira Souza C, Ojcius DM, Burnstock G (2005) P2X and P2Y purinergic receptors on human intestinal epithelial carcinoma cells: effects of extracellular nucleotides on apoptosis and cell proliferation. Am J Physiol Gastrointest Liver Physiol 288(5): G1024–G1035
32. Cressman VL, Lazarowski E, Homolya L, Boucher RC, Koller BH, Grubb BR (1999) Effect of loss of P2Y(2) receptor gene expression on nucleotide regulation of murine epithelial Cl(-) transport. J Biol Chem 274(37):26461–26468
33. Eltzschig HK, Ibla JC, Furuta GT, Leonard MO, Jacobson KA, Enjyoji K, Robson SC, Colgan SP (2003) Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in post-hypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. J Exp Med 198(5):783–796
34. Evers BM, Ishizuka J, Townsend CM Jr, Thompson JC (1994) The human carcinoid cell line, BON. A model system for the study of carcinoid tumors. Ann N Y Acad Sci 733:393–406
35. Evers BM, Townsend CM Jr, Upp JR, Allen E, Hurlbut SC, Kim SW, Rajaraman S, Singh P, Reubi JC, Thompson JC (1991) Establishment and characterization of a human carcinoid in nude mice and effect of various agents on tumor growth. Gastroenterology 101(2):303–311
36. Facer P, Knowles CH, Tam PK, Ford AP, Dyer N, Baecker PA, Anand P (2001) Novel capsaicin (VR1) and purinergic (P2X3) receptors in Hirschsprung's intestine. J Pediatr Surg 36(11):1679–1684
37. Fang X, Hu HZ, Gao N, Liu S, Wang GD, Wang XY, Xia Y, Wood JD (2006) Neurogenic secretion mediated by the purinergic P2Y1 receptor in guinea-pig small intestine. Eur J Pharmacol 536 (1-2):113–122
38. Furness JB, Kunze WA, Clerc N (1999) Nutrient tasting and signaling mechanisms in the gut. II. The intestine as a sensory organ: neural, endocrine, and immune responses. Am J Physiol 277(5 Pt 1):G922–G928
39. Furness JB (2000) Types of neurons in the enteric nervous system. J Auton Nerv Syst 81(1-3):87–96
40. Galligan JJ (2004) Enteric P2X receptors as potential targets for drug treatment of the irritable bowel syndrome. Br J Pharmacol 141:1294–1302
41. Gao N, Hu HZ, Zhu MX, Fang X, Liu S, Gao C, Wood JD (2006) The P2Y purinergic receptor expressed by enteric neurones in guinea-pig intestine. Neurogastroenterol Motil 18(4):316–323
42. Gerber JG, Payne NA (1988) Endogenous adenosine modulates gastric acid secretion to histamine in canine parietal cells. J Pharmacol Exp Ther 244(1):190–194
43. Groschel-Stewart U, Bardini M, Robson T, Burnstock G (1999) P2X receptors in the rat duodenal villus. Cell Tissue Res 297 (1):111–117
44. Gustafsson BI, Bakke I, Tommeras K, Waldum HL (2006) A new method for visualization of gut mucosal cells, describing the enterochromaffin cell in the rat gastrointestinal tract. Scand J Gastroenterol 41:390–395
45. Guzman J, Yu JG, Suntres Z, Bozarov A, Cooke H, Javed N, Auer H, Palatini J, Hassanain HH, Cardounel AJ, Javed A, Grants I, Wunderlich JE, Christofi FL (2006) ADOA3R as a therapeutic target in experimental colitis: proof by validated high-density oligonucleotide microarray analysis. Inflamm Bowel Dis 12(8):766–789

46. Gwynne RM, Bornstein JC (2007) Local inhibitory reflexes excited by mucosal application of nutrient amino acids in guinea pig jejunum. Am J Physiol Gastrointest Liver Physiol 292(6): G1660–G1670
47. Hede SE, Amstrup J, Christoffersen BC, Novak I (1999) Purinoceptors evoke different electrophysiological responses in pancreatic ducts. P2Y inhibits K(+) conductance, and P2X stimulates cation conductance. J Biol Chem 274(45):31784–31791
48. Heldsinger AA, Vinik AI, Fox IH (1986) Inhibition of guinea-pig oxyntic cell function by adenosine and prostaglandins. J Pharmacol Exp Ther 237(2):351–356
49. Hu HZ, Gao N, Lin Z, Gao C, Liu S, Ren J, Xia Y, Wood JD (2001) P2X(7) receptors in the enteric nervous system of guinea-pig small intestine. J Comp Neurol 440(3):299–310
50. Hu HZ, Gao N, Zhu MX, Liu S, Ren J, Gao C, Xia Y, Wood JD (2003) Slow excitatory synaptic transmission mediated by P2Y1 receptors in the guinea-pig enteric nervous system. J Physiol 550 (Pt 2):493–504
51. Hubel KA (1978) The effects of electrical field stimulation and tetrodotoxin on ion transport by the isolated rabbit ileum. J Clin Invest 62(5):1039–1047
52. Ishiguro H, Naruse S, Kitagawa M, Hayakawa T, Case RM, Steward MC (1999) Luminal ATP stimulates fluid and HCO3- secretion in guinea-pig pancreatic duct. J Physiol 519(Pt 2):551–558
53. Jacobson KA, Gao ZG (2006) Adenosine receptors as therapeutic targets. Nat Rev Drug Discov 5(3):247–264
54. Jenkinson KM, Reid JJ (2000) The P(2)-purinoceptor antagonist suramin is a competitive antagonist at vasoactive intestinal peptide receptors in the rat gastric fundus. Br J Pharmacol 130 (7):1632–1638
55. Jijon HB, Walker J, Hoentjen F, Diaz H, Ewaschuk J, Jobin C, Madsen KL (2005) Adenosine is a negative regulator of NF-kappaB and MAPK signaling in human intestinal epithelial cells. Cell Immunol 237(2):86–95
56. Kerstan D, Gordjani N, Nitschke R, Greger R, Leipziger J (1998) Luminal ATP induces K^+ secretion via a P2Y2 receptor in rat distal colonic mucosa. Pflugers Arch 436(5):712–716
57. Khakh BS, Burnstock G, Kennedy C, King BF, North RA, Seguela P, Voigt M, Humphrey PP (2001) International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol Rev 53(1):107–118
58. Kidd M, Modlin IM, Eick GN, Champaneria MC (2006) Isolation, functional characterization, and transcriptome of Mastomys ileal enterochromaffin cells. Am J Physiol Gastrointest Liver Physiol 291(5):G778–G791
59. Kim M, Cooke HJ, Javed NH, Carey HV, Christofi F, Raybould HE (2001) D-glucose releases 5-hydroxytryptamine from human BON cells as a model of enterochromaffin cells. Gastroenterology 121(6):1400–1406
60. Kimura Y, Turner JR, Braasch DA, Buddington RK (2005) Lumenal adenosine and AMP rapidly increase glucose transport by intact small intestine. Am J Physiol Gastrointest Liver Physiol 289(6):G1007–G1014
61. Kolachala VL, Bajaj R, Chalasani M, Sitaraman SV (2008) Purinergic receptors in gastrointestinal inflammation. Am J Physiol Gastrointest Liver Physiol 294(2):G401–G410
62. Konig J, Schreiber R, Mall M, Kunzelmann K (2002) No evidence for inhibition of ENaC through CFTR-mediated release of ATP. Biochim Biophys Acta 1565(1):17–28
63. Konturek SJ, Brzozowski T, Pajdo R, Konturek PC, Kwiecien S, Sliwowski Z, Pawlik M, Ptak A, Drozdowicz D, Hahn EG (2001) Gastric preconditioning induced by short ischemia: the role of prostaglandins, nitric oxide and adenosine. Med Sci Monit 7(4):610–621
64. Kwok YN, McIntosh C, Brown J (1990) Augmentation of release of gastric somatostatin-like immunoreactivity by adenosine, adenosine triphosphate and their analogs. J Pharmacol Exp Ther 255(2):781–788
65. Lazarowski ER, Rochelle LG, O'Neal WK, Ribeiro CM, Grubb BR, Zhang V, Harden TK, Boucher RC (2001) Cloning and functional characterization of two murine uridine nucleotide receptors reveal a potential target for correcting ion transport deficiency in cystic fibrosis gallbladder. J Pharmacol Exp Ther 297(1):43–49
66. Lee MG, Schultheis PJ, Yan M, Shull GE, Bookstein C, Chang E, Tse M, Donowitz M, Park K, Muallem S (1998) Membrane-limited expression and regulation of Na^+-H^+ exchanger isoforms by P2 receptors in the rat submandibular gland duct. J Physiol 513(Pt 2):341–357
67. Linden DR, Chen JX, Gershon MD, Sharkey KA, Mawe GM (2003) Serotonin availability is increased in mucosa of guinea pigs with TNBS-induced colitis. Am J Physiol Gastrointest Liver Physiol 285(1):G207–G216
68. Lomax AE, O'Reilly M, Neshat S, Vanner SJ (2007) Sympathetic vasoconstrictor regulation of mouse colonic submucosal arterioles is altered in experimental colitis. J Physiol 583 (Pt 2):719–730
69. Lomax AE, Linden DR, Mawe GM, Sharkey KA (2006) Effects of gastrointestinal inflammation on enteroendocrine cells and enteric neural reflex circuits. Auton Neurosci 126–127:250–257
70. Lomax AE, Fernández E, Sharkey KA (2005) Plasticity of the enteric nervous system during intestinal inflammation. Neurogastroenterol Motil 17(1):4–15
71. Lomax AE, Furness JB (2000) Neurochemical classification of enteric neurons in the guinea-pig distal colon. Cell Tissue Res 302(1):59–72
72. Luo X, Zheng W, Yan M, Lee MG, Muallem S (1999) Multiple functional P2X and P2Y receptors in the luminal and basolateral membranes of pancreatic duct cells. Am J Physiol 277(2 Pt 1): C205–C215
73. Madara JL, Nash S, Parkos C (1991) Neutrophil-epithelial cell interactions in the intestine. Adv Exp Med Biol 314:329–334
74. Matos JE, Sorensen MV, Geyti CS, Robaye B, Boeynaems JM, Leipziger J (2007) Distal colonic Na(+) absorption inhibited by luminal P2Y(2) receptors. Pflugers Arch 454(6):977–987
75. Matos JE, Robaye B, Boeynaems JM, Beauwens R, Leipziger J (2005) K^+ secretion activated by luminal P2Y2 and P2Y4 receptors in mouse colon. J Physiol 564(Pt 1):269–279
76. McAlroy HL, Ahmed S, Day SM, Baines DL, Wong HY, Yip CY, Ko WH, Wilson SM, Collett A (2000) Multiple P2Y receptor subtypes in the apical membranes of polarized epithelial cells. Br J Pharmacol 131(8):1651–1658
77. Naganuma M, Wiznerowicz EB, Lappas CM, Linden J, Worthington MT, Ernst PB (2006) Cutting edge: critical role for A2A adenosine receptors in the T cell-mediated regulation of colitis. J Immunol 177(5):2765–2769
78. Nahum V, Zundorf G, Levesque SA, Beaudoin AR, Reiser G, Fischer B (2002) Adenosine 5′-O (1-boranotriphosphate) derivatives as novel P2Y(1) receptor agonists. J Med Chem 45 (24):5384–5396
79. Namiot Z, Rutkiewicz J, Stasiewicz J, Gorski J (1990) Adenosine deaminase activity in the human gastric mucosa in relation to acid secretion. Digestion 45(3):172–175
80. Namiot Z, Rutkiewicz J, Stasiewicz J, Baranczuk E, Marcinkiewicz M (1991) Adenosine deaminase activity in the gastric mucosa in patients with gastric ulcer. Effects of ranitidine and sucralfate. Eur J Pharmacol 205(1):101–103
81. Nguyen TD, Moody MW, Savard CE, Lee SP (1998) Secretory effects of ATP on nontransformed dog pancreatic duct epithelial cells. Am J Physiol 275(1 Pt 1):G104–G113

82. Odashima M, Bamias G, Rivera-Nieves J, Linden J, Nast CC, Moskaluk CA, Marini M, Sugawara K, Kozaiwa K, Otaka M, Watanabe S, Cominelli F (2005) Activation of A2A adenosine receptor attenuates intestinal inflammation in animal models of inflammatory bowel disease. Gastroenterology 129(1):26–33
83. Odashima M, Otaka M, Jin M, Komatsu K, Wada I, Horikawa Y, Matsuhashi T, Hatakeyama N, Oyake J, Ohba R, Watanabe S, Linden J (2006) Attenuation of gastric mucosal inflammation induced by aspirin through activation of A2A adenosine receptor in rats. World J Gastroenterol 12(4):568–573
84. Pan H, Gershon MD (2000) Activation of intrinsic afferent pathways in submucosal ganglia of the guinea pig small intestine. J Neurosci 20(9):3295–3309
85. Poole DP, Castelucci P, Robbins HL, Chiocchetti R, Furness JB (2002) The distribution of P2X3 purine receptor subunits in the guinea pig enteric nervous system. Auton Neurosci 101(1-2):39–47
86. Puurunen J, Ruoff HJ, Schwabe U (1987) Lack of direct effect of adenosine on the parietal cell function in the rat. Pharmacol Toxicol 60(4):315–317
87. Raybould HE, Cooke HJ, Christofi FL (2004) Sensory mechanisms: transmitters, modulators and reflexes. Neurogastroenterol Motil 16(Suppl 1):60–63
88. Reed DE, Vanner S (2007) Mucosal stimulation activates secretomotor neurons via long myenteric pathways in guinea pig ileum. Am J Physiol Gastrointest Liver Physiol 292(2):G608–G614
89. Rivkees SA, Reppert SM (1992) RFL9 encodes an A2b-adenosine receptor. Mol Endocrinol 6(10):1598–1604
90. Robaye B, Ghanem E, Wilkin F, Fokan D, Van Driessche W, Schurmans S, Boeynaems JM, Beauwens R (2003) Loss of nucleotide regulation of epithelial chloride transport in the jejunum of P2Y4-null mice. Mol Pharmacol 63(4):777–783
91. Rodrigues RJ, Almeida T, Richardson PJ, Oliveira CR, Cunha RA (2005) Dual presynaptic control by ATP of glutamate release via facilitatory P2X1, P2X2/3, and P2X3 and inhibitory P2Y1, P2Y2, and/or P2Y4 receptors in the rat hippocampus. J Neurosci 25(27):6286–6295
92. Roman RM, Fitz JG (1999) Emerging roles of purinergic signaling in gastrointestinal epithelial secretion and hepatobiliary function. Gastroenterology 116(4):964–979
93. Ruan HZ, Burnstock G (2004) P2X2 and P2X3 receptor expression in the gallbladder of the guinea pig. Auton Neurosci 111(2):89–96
94. Ruan HZ, Burnstock G (2005) The distribution of P2X5 purinergic receptors in the enteric nervous system of mouse. Cell Tissue Res 319(2):191–200
95. Ruhl A, Nasser Y, Sharkey KA (2004) Enteric glia. Neurogastroenterol Motil 16(Suppl 1):44–49
96. Salter KD, Fitz JG, Roman RM (2000) Domain-specific purinergic signaling in polarized rat cholangiocytes. Am J Physiol Gastrointest Liver Physiol 278(3):G492–G500
97. Saslow SB, Scolapio JS, Camilleri M, Forstrom LA, Thomforde GM, Burton DD, Rubin J, Pitot HC, Zinsmeister AR (1998) Medium-term effects of a new 5HT3 antagonist, alosetron, in patients with carcinoid diarrhoea. Gut 42(5):628–634
98. Sanchez-Pozo A, Gil A (2002) Nucleotides as semiessential nutritional components. Br J Nutr 87(Suppl 1):S135–S137
99. Sarwar G, Brule D (1991) Assessment of the uricogenic potential of processed foods based on the nature and quantity of dietary purines. Prog Food Nutr Sci 15(3):159–181
100. Schlenker T, Romac JM, Sharara AI, Roman RM, Kim SJ, LaRusso N, Liddle RA, Fitz JG (1997) Regulation of biliary secretion through apical purinergic receptors in cultured rat cholangiocytes. Am J Physiol 273(5 Pt 1):G1108–G1117
101. Schreiber R, Konig J, Sun J, Markovich D, Kunzelmann K (2003) Effects of purinergic stimulation, CFTR and osmotic stress on amiloride-sensitive Na+ transport in epithelia and Xenopus oocytes. J Membr Biol 192(2):101–110
102. Schwiebert EM, Egan ME, Hwang TH, Fulmer SB, Allen SS, Cutting GR, Guggino WB (1995) CFTR regulates outwardly rectifying chloride channels through an autocrine mechanism involving ATP. Cell 81(7):1063–1073
103. Sitaraman SV, Merlin D, Wang L, Wong M, Gewirtz AT, Si-Tahar M, Madara JL (2001) Neutrophil-epithelial crosstalk at the intestinal lumenal surface mediated by reciprocal secretion of adenosine and IL-6. J Clin Invest 107(7):861–869
104. Strohmeier GR, Lencer WI, Patapoff TW, Thompson LF, Carlson SL, Moe SJ, Carnes DK, Mrsny RJ, Madara JL (1997) Surface expression, polarization, and functional significance of CD73 in human intestinal epithelia. J Clin Invest 99(11):2588–2601
105. Suarez-Huerta N, Pouillon V, Boeynaems J, Robaye B (2001) Molecular cloning and characterization of the mouse P2Y4 nucleotide receptor. Eur J Pharmacol 416(3):197–202
106. Sundaram U, Hassanain H, Suntres Z, Yu JG, Cooke HJ, Guzman J, Christofi FL (2003) Rabbit chronic ileitis leads to up-regulation of adenosine A1/A3 gene products, oxidative stress, and immune modulation. Biochem Pharmacol 65 (9):1529–1538
107. Vank C, Fromter E, Kottra G (1999) The activation of an apical Cl- conductance by extracellular ATP is potentiated by genistein in Necturus gallbladder epithelium. Pflugers Arch 438(4):497–501
108. Van Nassauw L, Brouns I, Adriaensen D, Burnstock G, Timmermans JP (2002) Neurochemical identification of enteric neurons expressing P2X(3) receptors in the guinea-pig ileum. Histochem Cell Biol 118(3):193–203
109. Van Nassauw L, Costagliola A, Van Op den Bosch J, Cecio A, Vanderwinden JM, Burnstock G, Timmermans JP (2006) Region-specific distribution of the P2Y4 receptor inenteric glial cells and interstitial cells of Cajal within the guinea-pig gastrointestinal tract. Auton Neurosci 126-127:299–306
110. von Kugelgen I (2006) Pharmacological profiles of cloned mammalian P2Y-receptor subtypes. Pharmacol Ther 110(3):415–432
111. Vulchanova L, Arvidsson U, Riedl M, Wang J, Buell G, Surprenant A, North RA, Elde R (1996) Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. Proc Natl Acad Sci U S A 93(15):8063– 8067
112. Weber E, Neunlist M, Schemann M, Frieling T (2001) Neural components of distension-evoked secretory responses in the guinea-pig distal colon. J Physiol 536(Pt 3):741–751
113. Wood JD (2006) The enteric purinergic P2Y(1) receptor. Curr Opin Pharmacol 6(6):564–570
114. Wunderlich J, Xue J, Cooke HJ, Christofi FL (2004) Visualization and mapping of mechanosensitive P2X receptors in the human BON–enterochromaffin cell model. Gastroenterology 126 (Suppl 2):A–412
115. Wunderlich JE, Needleman BJ, Chen Z, Yu JG, Wang YZ, Grants I, Mikami D, Melvin WS, Cooke HJ, Christofi FL (2008) Dual purinergic synaptic transmission in the human enteric nervous system. Am J Physiol Gastrointest Liver Physiol 294(2): G554–G566
116. Wynn G, Rong W, Xiang Z, Burnstock G (2003) Purinergic mechanisms contribute to mechanosensory transduction in the rat colorectum. Gastroenterology 125(5):1398–1409
117. Xiang Z, Burnstock G (2006) Distribution of P2Y(6) and P2Y (12) receptor: their colocalization with calbindin, calretinin and nitric oxide synthase in the guinea pig enteric nervous system. Histochem Cell Biol 125(4):327–336
118. Xiang Z, Burnstock G (2005) Distribution of P2Y2 receptors in the guinea pig enteric nervous system and its coexistence with P2X2 and P2X3 receptors, neuropeptide Y, nitric oxide synthase and calretinin. Histochem Cell Biol 124(5):379–390

119. Xiang Z, Burnstock G (2004) P2X2 and P2X3 purinoceptors in the rat enteric nervous system. Histochem Cell Biol 121(3):169–179
120. Xue J Christofi FL, Cooke HJ (2007) P2Y12 receptors inhibit mechanosensitive release of 5-HT from human carcinoid BON cells. Gastroenterology 132:A547
121. Xue J, Christofi FL, Cooke HJ (2007) Mechanosensitive release of calcium or 5HT: P2Y1 receptor over-expression in human Bon cells. Gastroenterology 132(Suppl 2):A–242
122. Yiangou Y, Facer P, Baecker PA, Ford AP, Knowles CH, Chan CL, Williams NS, Anand P (2001) ATP-gated ion channel P2X (3) is increased in human inflammatory bowel disease. Neurogastroenterol Motil 13(4):365–369
123. Yip L, Kwok YN (2004) Role of adenosine A2A receptor in the regulation of gastric somatostatin release. J Pharmacol Exp Ther 309(2):804–815
124. Yip L, Leung HC, Kwok YN (2004) Effect of omeprazole on gastric adenosine A1 and A2A receptor gene expression and function. J Pharmacol Exp Ther 311(1):180–189
125. Yip L, Leung HC, Kwok YN (2004) Role of adenosine A1 receptor in the regulation of gastrin release. J Pharmacol Exp Ther 310(2):477–487